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CONTENTS

No. 1. June 30, 1917

PAGE

MACFIE, J. W. S.; and INGRAM, A.

Observations on Malaria in the Gold Coast Colony, West Africa 1

CARTER, HENRY F.; MACKINNON, DORIS L.; MATTHEWS, J. R.; and SMITH, A. MALINS. Part V with STEPHENS, Lieut.-Col. J. W. W.

Protozoological Investigation of Cases of Dysentery Conducted at the Liverpool School of Tropical Medicine. (Second Report) 27

YORKE, WARRINGTON; and BLACKLOCK, B.

The Occurrence of *Ankylostoma ceylanicum* in West African Dogs 69

MACFIE, J. W. S.; and CARTER, HENRY F.

The Occurrence of *Spirochaeta eurygyrata* in Europeans in England, with a Note on a Second Species of *Spirochaeta* from the Human Intestine ... 75

MACFIE, J. W. S.; and YORKE, WARRINGTON.

The Relapsing Fever Spirochaetes 81

YORKE, WARRINGTON; CARTER, HENRY F.; MACKINNON, DORIS L.; MATTHEWS, J. R.; and SMITH, A. MALINS.

Persons who have never been out of Great Britain as Carriers of *Entamoeba histolytica* 87

STEPHENS, Lieut.-Col. J. W. W.; YORKE, W.; BLACKLOCK, B.; MACFIE, J. W. S.; and COOPER, C. FORSTER.

Studies in the Treatment of Malaria: I.—Intravenous Injections of Tartar Emetic 91

STEPHENS, Lieut.-Col. J. W. W.; YORKE, W.; BLACKLOCK, B.; MACFIE, J. W. S.; and COOPER, C. FORSTER.

Studies in the Treatment of Malaria: II.—Intramuscular Injections of Quinine Bihydrochloride in Simple Tertian Malaria 113

338243

CONTENTS

No. 2. August 23, 1917

PAGE

YORKE, WARRINGTON ; and BLACKLOCK, B.

Observations on the Periodicity of *Microfilaria nocturna* 127

STEPHENS, Lieut.-Col. J. W. W. ; YORKE, W. ; BLACKLOCK, B. ; MACFIE, J. W. S. ; and COOPER, C. FORSTER.

Studies in the Treatment of Malaria : III.—Intravenous Injections of Quinine Bihydrochloride 149

STEPHENS, Lieut.-Col. J. W. W. ; YORKE, W. ; BLACKLOCK, B. ; MACFIE, J. W. S. ; and COOPER, C. FORSTER.

Studies in the Treatment of Malaria : IV.—Intramuscular Injections of Amylopsin and Trypsin in Simple Tertian Malaria 165

STEPHENS, Lieut.-Col. J. W. W. ; YORKE, W. ; BLACKLOCK, B. ; MACFIE, J. W. S. ; COOPER, C. FORSTER ; and CARTER, HENRY F.

Studies in the Treatment of Malaria : V.—Intramuscular Injections of Quinine Alkaloid in Simple Tertian Malaria 173

SMITH, A. MALINS ; and MATTHEWS, J. R.

Further Records of the Occurrence of Intestinal Protozoa in Non-Dysenteric Cases 183

CARTER, HENRY F. ; and MATTHEWS, J. R.

The Value of Concentrating the Cysts of Protozoal Parasites in Examining the Stools of Dysenteric Patients for Pathogenic Entamoebae 195

COGHILL, H. SINCLAIR.

Some Results obtained by Heiser's Treatment of Leprosy in Nigeria ... 205

CONTENTS

No. 3. January 31, 1918

PAGE

CHALMERS, ALBERT J.; and PEKKOLA, WAINÖ.

Chilomastix mesnili (Wenyon, 1910) 213

DE KORTE, W. E.

Nocardia cylindracea: A South African Otomycosis 265

CHRISTY, CUTHBERT.

Tsetse Flies and Fly-Belts 279

STEPHENS, Lieut.-Col. J. W. W.; YORKE, W.; BLACKLOCK, B.; MACFIE, J. W. S.; COOPER, C. FORSTER; and CARTER, HENRY F.

Studies in the Treatment of Malaria: VI.—Oral Administration of Quinine for Two Consecutive Days only, in Simple Tertian Malaria 283

CONTENTS

No. 4. May 11, 1918

PAGE

STEPHENS, Lieut.-Col. J. W. W.; YORKE, W.; BLACKLOCK, B.; MACFIE, J. W. S.; COOPER, C. FORSTER; and CARTER, HENRY F.

Studies in the Treatment of Malaria: VII.—Oral Administration of Quinine Sulphate Daily over Prolonged Periods, in Simple Tertian Malaria 309

STEPHENS, Lieut.-Col. J. W. W.; YORKE, W.; BLACKLOCK, B.; MACFIE, J. W. S.; COOPER, C. FORSTER; and CARTER, HENRY F.

Studies in the Treatment of Malaria: VIII.—Oral Administration of Quinine Sulphate for Two Consecutive Days Weekly over Prolonged Periods, in Simple Tertian Malaria 331

STEPHENS, Lieut.-Col. J. W. W.; YORKE, W.; BLACKLOCK, B.; MACFIE, J. W. S.; COOPER, C. FORSTER; and CARTER, HENRY F.

Studies in the Treatment of Malaria: IX.—A Comparison of the Results of Interrupted and Continuous Quinine Administration 359

SCHWETZ, Dr. J.

A Comparative Study of the Habits of *Glossina brevipalpis*, Newst., *G. fusca*, West., and *G. pallidipes*, Aust., in the Belgian Congo 365

YORKE, WARRINGTON; and MACFIE, J. W. S.

Strongylidae in Horses: I.—*Cylicostomum longibursatum* sp. n. 399

YORKE, WARRINGTON; and MACFIE, J. W. S.

Strongylidae in Horses: II.—*Cylicostomum minutum* sp. n. 405

YORKE, WARRINGTON; and MACFIE, J. W. S.

Strongylidae in Horses: III.—*Cylicostomum nassatum*, Looss. var. *parvum* ... 411

STEPHENS, Lieut.-Col. J. W. W.; YORKE, W.; BLACKLOCK, B.; MACFIE, J. W. S.; COOPER, C. FORSTER; and CARTER, HENRY F.

Studies in the Treatment of Malaria: X.—Oral Administration of Quinine Sulphate Grains 120 on Two Consecutive Days Only, in Simple Tertian Malaria 417

STEPHENS, Lieut.-Col. J. W. W.; YORKE, W.; BLACKLOCK, B.; MACFIE, J. W. S.; COOPER, C. FORSTER; and CARTER, HENRY F.

Studies in the Treatment of Malaria: XI.—Oral Administration of Quinine Sulphate Grains 90 on Two Consecutive Days Weekly over a Period of Three Weeks, in Simple Tertian Malaria 421

STEPHENS, Lieut.-Col. J. W. W.; YORKE, W.; BLACKLOCK, B.; MACFIE, J. W. S.; COOPER, C. FORSTER; and CARTER, HENRY F.

Studies in the Treatment of Malaria: XII.—At what time after Cessation of Quinine Treatment do Relapses Occur in Simple Tertian Malaria? 425

RAMSDEN, W.; and LIPKIN, I. J.

Detection and Estimation of Quinine in Blood and Urine 443

INDEX

INDEX

PAGE

INDEX OF AUTHORS iii

GENERAL INDEX iv

INDEX OF SPECIES AND VARIETIES NEW TO SCIENCE x

INDEX OF AUTHORS

PAGE

Blacklock, B.; Stephens, J. W. W.; Yorke, W.; Macfie, J. W. S.; Cooper, C. F.; Carter, H. F. 91, 113, 149, 165, 173, 283, 309, 331, 359, 417, 421, 425

Blacklock, B.; and Yorke, W. 69, 127

Carter, H. F.; and Macfie, J. W. S. 75

Carter, H. F.; Mackinnon, D. L.; Matthews, J. R.; and Smith, A. M. 27

Carter, H. F.; and Matthews, J. R. 195

Carter, H. F.; Stephens, J. W. W.; Yorke, W.; Blacklock, B.; Macfie, J. W. S.; and Cooper, C. F. 173, 283, 309, 331, 359, 417, 421, 425

Carter, H. F.; Yorke, W.; Mackinnon, D. L.; Matthews, J. R.; and Smith, A. M. 87

Chalmers, A. J.; and Pekkola, W. ... 213

Christy, C. 279

Coghill, H. S. 205

Cooper, C. F.; Stephens, J. W. W.; Yorke, W.; Blacklock, B.; Macfie, J. W. S.; and Carter, H. F. 91, 113, 149, 165, 173, 283, 309, 331, 359, 417, 421, 425

Ingram, A.; and Macfie, J. W. S. ... 1

de Korté, W. E. 265

Lipkin, I. J.; and Ramsden, W. 443

Macfie, J. W. S.; and Carter, H. F. 75

Macfie, J. W. S.; and Ingram, A. ... 1

Macfie, J. W. S.; Stephens, J. W. W.; Yorke, W.; Blacklock, B.; Cooper, C. F.; Carter, H. F. 91, 113, 149, 165, 173, 283, 309, 331, 359, 417, 421, 425

Macfie, J. W. S.; and Yorke, W. 81, 399 405, 411

Mackinnon, D. L.; Carter, H. F.; Matthews, J. R.; and Smith, A. M. 27

PAGE

Mackinnon, D. L.; Yorke, W.; Carter, H. F.; Matthews, J. R.; and Smith, A. M. 87

Matthews, J. R.; and Carter, H. F. 195

Matthews, J. R.; Carter, H. F.; Mackinnon, D. L.; and Smith, A. M. 27

Matthews, J. R.; and Smith, A. M. 183

Matthews, J. R.; Yorke, W.; Carter, H. F.; Mackinnon, D. L.; and Smith, A. M. 87

Pekkola, W.; and Chalmers, A. J. ... 213

Ramsden, W.; and Lipkin, I. J. 443

Schwetz, J. 365

Smith, A. M.; Carter, H. F.; Mackinnon, D. L.; and Matthews, J. R. 27

Smith, A. M.; and Matthews, J. R.... 183

Smith, A. M.; Yorke, W.; Carter, H. F.; Mackinnon, D. L.; and Matthews, J. R. 87

Stephens, J. W. W.; Yorke, W.; Blacklock, B.; Macfie, J. W. S.; Cooper, C. F.; and Carter, H. F. 91, 113, 149, 165, 173, 283, 309, 331, 359, 417, 421, 425

Yorke, W.; and Blacklock, B.....69, 127

Yorke, W.; Carter, H. F.; Mackinnon, D. L.; Matthews, J. R.; and Smith, A. M. 87

Yorke, W.; and Macfie, J. W. S. 81, 399, 405, 411

Yorke, W.; Stephens, J. W. W.; Blacklock, B.; Macfie, J. W. S.; Cooper, C. F.; and Carter, H. F. 91, 113, 149, 165, 173, 283, 309, 331, 359, 417, 421, 425

PAGE

Accra, Seasonal incidence of malaria at 5
Alcresta ipecac. in *E. coli* infections ... 40
" " *E. histolytica* infections 64
Amylopsin and trypsin in malaria ... 165
Anaplasma-like bodies in blood of malarial patients 21
Ankylostoma caninum 69
" *ceylanicum* in West African dogs 69
" *duodenale* 192, 200
Antimony in malaria 92
Ascaris lumbricoides 15
Blacklock, B., Stephens, J. W. W., Yorke, W., Macfie, J. W. S., Cooper, C. F., and Carter, H. F. Studies in the Treatment of Malaria, 91, 113, 149, 165, 173, 283, 309, 331, 359, 417, 421, 425
Blacklock, B., and Yorke, W. Observations on the Periodicity of *Microfilaria nocturna* 127
Blacklock, B., and Yorke, W. The occurrence of *Ankylostoma ceylanicum* in West African dogs 69
Blackwater fever, Peculiar malaria parasites in 22
Blastocystis hominis, associated with *C. mesnili* 214
Blood, Detection and estimation of quinine in 443
Blood, Periodicity of *M. nocturna* in the 128
Callodictyon, Genus 236, 248, 253
Carriers of *E. histolytica*, Healthy individuals as 187, 188
" " Persons who have never been out of Great Britain as 87
Carter, H. F., and Macfie, J. W. S. The occurrence of *Spirochaeta eurygyrata* in Europeans in England, with a note on a second species of *Spirochaeta* from the human intestine 75

PAGE

Carter, H. F., Mackinnon, D. L., Matthews, J. R., and Smith, A. M. Protozoological investigation of cases of dysentery conducted at the Liverpool School of Tropical Medicine. (Second Report) 27
Carter, H. F., and Matthews, J. R. The value of concentrating the cysts of protozoal parasites in examining the stools of dysenteric patients for pathogenic entamoebae 195
Carter, H. F., Stephens, J. W. W., Yorke, W., Blacklock, B., Macfie, J. W. S., and Cooper, C. F. Studies in the Treatment of Malaria, 173, 283, 309, 331, 359, 417, 421, 425
Carter, H. F., Yorke, W., Mackinnon, D. L., Matthews, J. R., and Smith, A. M. Persons who have never been out of Great Britain as carriers of *Entamoeba histolytica* 87
Chalmers, A. J., and Pekkola, W. *Chilomastix mesnili* 213
Chaulmoogra oil in leprosy 205
Chilomastix, Genus ...241, 249, 253, 254
" Species of 254
" Synonyms of 254
" *bettencourti* 255, 256
" *bocis* 255
" *caprae* 255, 256
" *caulleryi*, 218, 242, 254, 256
" *cuniculi* 255, 256
" *intestinalis* 255
" *mesnili* 213
" " associated with *E. coli* 29
" " associated with *E. coli* and *G. intestinalis* 30
" " associated with *E. coli* and *T. intestinalis* 30
" " associated with *E. histolytica* and *E. coli* 30
" " associated with *E. histolytica*, *E. coli* and *G. intestinalis* 30

PAGE

Chilomastix mesnili, associated with *G. intestinalis* 29
„ „ Classification 231
„ „ Development 228
„ „ Geographical distribution 222
„ „ History 214
„ „ Infections with..45, 184
„ „ Morphology 224
„ „ Pathogenicity...214, 257
„ „ Synonyms of...254, 256
„ „ Treatment of infections with ... 257
„ „ Zoological distribution 222
„ *motellae* 254, 256
Chilomitus, Genus 245
Chloraster, Genus 234
Christy, C. Tsetse flies and fly-belts 279
Cimaenomonas, Genus 238
Coghill, H. S. Some results obtained by Heiser's treatment of leprosy in Nigeria 205
Concentration examinations for pathogenic protozoa 195
Congo, Comparative study of *Glossina* in the Belgian 365
Continuous quinine administration... 309
„ compared with interrupted quinine administration 359
Cooper, C. F., Stephens, J. W. W., Yorke, W., Blacklock, B., Macfie, J. W. S., and Carter, H. F. Studies in the Treatment of Malaria, 91, 113, 149, 165, 173, 283, 309, 331, 359, 417, 421, 425
Costia, Genus 239
Costiopsis, Genus 240, 249, 253
Cyathomastix, Genus 244
„ Synonym of *Chilomastix* 254
Cylicostomum longibursatum, sp.n..... 400
„ *minutum*, sp.n. 405
„ *nassatum*, Looss, var. *parvum*, var. n. 411
Dallengeria, Genus 238, 248, 253
Devescovina, Genus 240, 249
Difämus, Genus 243
„ Synonym of *Chilomastix* ... 254
Dysentery, *G. intestinalis* as possible causal organism of 190

PAGE

Dysentery, Protozoological investigation of cases of 27
Elvirea, Genus 239
Embadomonas, Genus 242, 250, 253
Emetine bismuth iodide in *E. histolytica* infections 65
„ in *E. coli* infections 40
Entamoeba coli associated with *C. mesnili* 29
„ „ associated with *C. mesnili* and *T. intestinalis* 30
„ „ associated with *E. histolytica* 29, 184
„ „ associated with *E. histolytica* and *C. mesnili* 30, 184
„ „ associated with *E. histolytica* and *G. intestinalis* 30, 184
„ „ associated with *E. histolytica*, *G. intestinalis* and *C. mesnili* 30
„ „ associated with *E. histolytica* and *T. intestinalis* 30
„ „ associated with *G. intestinalis* 29, 184
„ „ associated with *G. intestinalis* and *C. mesnili*..... 30
„ „ Infections with, 39, 51, 184
„ *histolytica* associated with *E. coli* 29, 184
„ „ associated with *E. coli* and *C. mesnili*.....30, 184
„ „ associated with *E. coli* and *G. intestinalis*..30, 184
„ „ associated with *E. coli*, *G. intestinalis* and *C. mesnili*..... 30
„ „ associated with *E. coli* and *T. intestinalis* ... 30
„ „ associated with *G. intestinalis* 29, 184
„ „ associated with *T. intestinalis* 29

PAGE

Entamoeba histolytica, Cases of, from France..... 59
" " Experimental infection with 88
" " Healthy individuals as carriers of 187, 188
" " Infections with, 43, 53, 183, 184
" " infections, relapse in 67
" " infections, Treatment of 63
" " Malarial patients as carriers of 188
" " Non-dysenteric patients as carriers of ... 189
" " Persons who have never been out of Gt. Britain as carriers of... 87
" " Regional classification of carriers of ... 191
" *nana*, associated with *C.* " *mesnili* 213
Entamoebae, Concentration examinations for pathogenic..... 195
Enteromonas, Genus 245, 250, 253
" *hominis* 214
Euflagellata, Diagnostic table of 232
Eutrichomastix, Genus, ...246, 251
Fanapepea, Genus 219, 242
" synonym of *Chilomastix*... 254
Fever, *see* Malaria, Blackwater, Relapsing.
Filaria bancrofti 127
Filariasis, Periodicity of *M. nocturna* in case of..... 127
" Urine in case of 138
Fly-belts, Tsetse flies and 279
France, Cases of amoebic dysentery from 59
Giardia intestinalis associated with *C. mesnili*.....29, 214
" " associated with *E. coli* 29, 184
" " associated with *E. coli and C. mesnili* 30

PAGE

Giardia intestinalis associated with *E. histolytica* 29, 184
" " associated with *E. histolytica* and *E. coli* 30, 184
" " associated with *E. histolytica*, *E. coli* and *C. mesnili* 30
" " Infections with, 35, 49, 184, 190
" " as possible causal organism of dysentery 190
Glossina and game 280, 381
" *brevipalpis*, Comparative study of the habits of 365
" " crepuscular period of activity, 369, 370, 388
" " distribution of sexes, 371, 373, 398
" " incidence governed by type of vegetation, 369, 371, 383, 392
" " manner of resting 372, 373,393
" " resting-places, 371, 372, 383
" *fusca*, Comparative study of the habits of 365
" " distribution of sexes, 375, 397, 398
" " incidence governed by type of vegetation, 369, 370, 375, 376, 383, 386, 392
" " manner of resting, 374, 393
" " nocturnal period of activity, 369, 370, 387, 388, 389
" " resting-places 374
" *morsitans* 279, 280
" " distribution of sexes 398
" " diurnal period of activity 380
" " incidence governed by type of vegetation 367, 392
" " manner of resting 393

PAGE

Glossina pallidipes, Comparative study of the habits of 365
" " distribution of sexes 374, 398
" " incidence governed by type of vegetation, 369, 371, 378, 383, 392
" " manner of resting, 373, 374, 393
" " period of activity 369, 370, 371, 393
" " resting-places 373
" *palpalis*, and pigs 281
" " diurnal period of activity 393
" " incidence governed by type of vegetation 367, 370, 392
" " manner of resting, 392, 393
" " resting-places 392
Gold Coast, Disease incidence in 1
" Malaria in 1
" Relative frequency of occurrence of different malaria parasites in ... 2
Heiser's treatment of leprosy, Results obtained by 205
Herapath test for quinine 444
Hexamastix, Genus 243, 244, 250
Horses, *Strongylidae* in ...399, 405, 411
'I bodies' in dysenteric cases 32
" non-dysenteric cases ... 192
India, Varieties of malaria in 4
Ingram, A., and Macfie, J. W. S. Observations on Malaria in the Gold Coast Colony, West Africa 1
Interrupted quinine administration, 331, 421
" compared with continuous quinine administration 359
Intestinal protozoa in non-dysenteric cases 183
" " Number of examinations recommended, ...56, 185
Intramuscular injections of amylopsin and trypsin in malaria 165

PAGE

Intramuscular injections of quinine alkaloid in malaria 173
" " quinine bihydrochloride in malaria 113
Intravenous injections of quinine bihydrochloride in malaria 149
" " tartar emetic in malaria 92
de Korté, W. E. *Nocardia cylindracea*: a South African otomycosis 265
Lamblia, see *Giardia*.
Leiper on *A. ceylanicum* 70
Leprosy, Heiser's treatment of 205
Lipkin, I. J., and Ramsden, W. Detection and estimation of quinine in blood and urine 443
Macfie, J. W. S., and Carter, H. F. The occurrence of *Spirochaeta eurygyrata* in Europeans in England, with a note on a second species of *Spirochaeta* from the human intestine 75
Macfie, J. W. S., and Ingram, A. Observations on Malaria in the Gold Coast Colony, West Africa..... 1
Macfie, J. W. S., Stephens, J. W. W., Yorke, W., Blacklock, B., Cooper, C. F., and Carter, H. F. Studies in the Treatment of Malaria, 91, 113, 149, 165, 173, 283, 309, 331, 359, 417, 421, 425
Macfie, J. W. S., and Yorke, W. The Relapsing Fever Spirochaetes 81
Macfie J. W. S., and Yorke, W. *Strongylidae* in Horses ...399, 405, 411
Mackinnon, D. L., Carter, H. F., Matthews, J. R., and Smith, A. M. Protozoological investigation of cases of dysentery conducted at the Liverpool School of Tropical Medicine (Second Report) 27
Mackinnon, D. L., Yorke, W., Carter, H. F., Matthews, J. R., and Smith, A. M. Persons who have never been out of Great Britain as carriers of *Entamoeba histolytica* 87
Macrostoma, Genus 216, 217, 241

PAGE

Malaria, Amylopsin and trypsin in ... 165
„ Observations on, in the Gold Coast Colony, West Africa 1
„ and oedema, Association of... 14
„ parasites, Abnormal forms of sub-tertian type 8
„ parasites, Relative frequency of occurrence, in Gold Coast 2
„ Quinine alkaloid in 173
„ „ bihydrochloride in, 113, 149, 287
„ Relapses in simple tertian ... 425
„ Studies in the treatment of, 91, 113, 149, 165, 173, 283, 309, 331, 359, 417, 421, 425
„ Tartar emetic in 92
„ Varieties of, in India 4
Malarial patients as carriers of *E. histolytica* 188
Mastigophora, Diagnostic table of ... 231
Matthews, J. R., and Carter, H. F. The value of concentrating the cysts of protozoal parasites in examining the stools of dysenteric patients for pathogenic entamoebae 195
Matthews, J. R., Carter, H. F., Mackinnon, D. L., and Smith, A. M. Protozoological investigation of cases of dysentery conducted at the Liverpool School of Tropical Medicine (Second Report) 27
Matthews, J. R., and Smith, A. M. Further records of the occurrence of intestinal protozoa in non-dysenteric cases 183
Matthews, J. R., Yorke, W., Carter, H. F., Mackinnon, D. L., and Smith, A. M. Persons who have never been out of Great Britain as carriers of *Entamoeba histolytica*..... 87
Methyl emetine in *E. histolytica* infections 65
Microfilaria nocturna, Periodicity of, in the blood... 128
„ „ Periodicity of, in the urine... 139
Monocercomonas, Genus 237, 248
Monozoa, Diagnostic table of..... 233
Nephritis in malaria 14
Nigeria, Cases of leprosy in 205

PAGE

Nocardia cylindracea 265
Octomitus hominis, associated with *C. mesnili* 214
Oedema, Association of malaria and... 14
Otomycosis, A South African 265
Pekkola, W., and Chalmers, A. J. *Chilomastix mesnili* 213
Pentatrichomonas, Genus 244, 250
Periodicity of *Microfilaria nocturna* ... 127
Pigs, association of, with sleeping sickness 281
Plasmodium falciparum 17, 18 110
„ *malariae* 16, 17, 18
„ *tenue* 10, 11, 12, 13, 14
„ *vivax* ...10, 11, 17, 18, 110
Polymastix, Genus 238, 249
Precipitants for quinine 445
Protomonadina, Diagnostic table of ... 232
Protozoa, Concentration examinations for pathogenic 195
Protozoal infections, Regional classification of cases having..... 191
Protozoological investigation of cases of dysentery 27
Protrichomonas, Genus 242, 250
Pyramimonas, Genus 236
Quinine alkaloid in malaria 173
„ bihydrochloride in malaria, 113, 149, 287
„ Detection and estimation of, in blood and urine 443
„ Gravimetric estimation of ... 452
„ Nephelometric estimation of 457
„ Precipitants for 445
„ sulphate in malaria, 284, 289, 309, 331, 417, 421
„ urethane..... 164
„ Volumetric estimation of..... 451
Ramsden, W., and Lipkin, I. J. Detection and estimation of quinine in blood and urine 443
Relapses in simple tertian malaria..... 425
Relapsing Fever spirochaetes..... 81
Schwetz, J. A comparative study of the habits of *Glossina brevipalpis*, Newst., *G. fusca*, West., and *G. pallidipes*, Aust. in the Belgian Congo 365
Sleeping sickness, possible association with pigs 281

PAGE

Smith, A. M., Carter, H. F., Mackinnon, D. L., and Matthews, J. R. Protozoological investigation of cases of dysentery conducted at the Liverpool School of Tropical Medicine (Second Report) 27

Smith, A. M., and Matthews, J. R. Further records of the occurrence of intestinal protozoa in non-dysenteric cases 183

Smith, A. M., Yorke, W., Carter, H. F., Mackinnon, D. L., and Matthews, J. R. Persons who have never been out of Great Britain as carriers of *Entamoeba histolytica* ... 87

South African otomycosis, A..... 265

Spirochaeta carteri 81, 82

„ „ Measurements of ... 83

„ *duttoni* 81, 82

„ „ Measurements of ... 83

„ *eurygyrata* 82

„ „ in Europeans in England 75

„ *marchouxi* 84

„ *novyi* 81, 84

„ *recurrentis* 81, 82

„ „ Measurements of 83

„ New species of intestinal 77

Spirochaetes of Relapsing Fever 81

Stephens, J. W. W., Yorke, W., Blacklock, B., Macfie, J. W. S., Cooper, C. F. and Carter, H. F. Studies in the Treatment of Malaria, 91, 113, 149, 165, 173, 283, 309, 331, 359, 417, 421, 425

Strongylidae in Horses 399, 405, 411

Studies in the Treatment of Malaria :—

I. Intravenous injections of tartar emetic..... 91

II. Intramuscular injections of quinine bihydrochloride in simple tertian malaria 113

III. Intravenous injections of quinine bihydrochloride..... 149

IV. Intramuscular injections of amylopsin and trypsin in simple tertian malaria 165

V. Intramuscular injections of quinine alkaloid in simple tertian malaria 173

PAGE

VI. Oral administration of quinine for two consecutive days only in simple tertian malaria ... 283

VII. Oral administration of quinine sulphate daily over prolonged periods in simple tertian malaria 309

VIII. Oral administration of quinine sulphate for two consecutive days weekly over prolonged periods in simple tertian malaria 331

IX. A comparison of the results of interrupted and continuous quinine administration 359

X. Oral administration of quinine sulphate, grains 120, on two consecutive days only in simple tertian malaria 417

XI. Oral administration of quinine sulphate, grains 90, on two consecutive days weekly over a period of three weeks in simple tertian malaria 421

XII. At what time after cessation of quinine treatment do relapses occur in simple tertian malaria? 425

Taenia saginata, detection of eggs, by concentration examination..... 200

Tanret's reagent as precipitant for quinine..... 445

Tartar emetic in malaria 92

Tetrachilomastix, Genus 246, 251, 253

Tetramitidae, Affinities 251

„ Definition of 233

„ Genera of 234, 247

„ sub-families 251

Tetramitidinae, Genera of 253

Tetramitus, Genus 217, 236, 248, 253

Tetraselmis, Genus 237

Tetratrichomastix, Genus 244, 250

Tetratrichomonas, Genus 241, 249

Thalleioquin test for quinine 443

Treatment of malaria, 91, 113, 149, 165, 173, 283, 309, 331, 359, 417, 421, 425

Trichiuris trichiura in dysenteric case 32

„ „ in non-dysenteric case..... 192

„ „ Detection of, by concentration examinations ... 200

PAGE

Trichocephalus dispar, see *Trichiuris trichiura*.
Trichomastix, Genus 239
Trichomonadinae, Diagnostic table of 259
Trichomonas, Genus 234, 248
„ *intestinalis*, associated with *C. mesnili* 214
„ „ associated with *E. coli* and *C. mesnili* 30
„ „ associated with *E. histolytica* 29
„ „ associated with *E. histolytica* and *E. coli* 30
„ „ Infections with 47
Trimastix, Genus 238, 248, 253
Trimitus, Genus 241
Trypsin and amylopsin in malaria..... 165
Tsetse flies and fly-belts..... 279
Uncinaria criniformis (*stenocephala*), 70, 73
Urine in case of Filariasis 138
„ Detection and estimation of quinine in 443
„ Isolation of quinine from 455
Wagner's reagent as precipitant for quinine 446

PAGE

Waskia, Genus 247
West Africa, Malaria in the Gold Coast Colony 1
„ African dogs, *Ankylostoma ceylanicum* in 69
Wild animals as reservoir for sleeping sickness 281
Yorke, W., and Blacklock, B. Observations on the Periodicity of *Microfilaria nocturna* 127
Yorke, W., and Blacklock, B. The occurrence of *Ankylostoma ceylanicum* in West African dogs 69
Yorke, W., Carter, H. F., Mackinnon, D. L., Matthews, J. R., and Smith, A. M. Persons who have never been out of Great Britain as carriers of *Entamoeba histolytica* 87
Yorke, W., and Macfie, J. W. S. The Relapsing Fever Spirochaetes..... 81
Yorke, W., and Macfie, J. W. S., *Strongylidae* in Horses, ...399, 405, 411
Yorke, W., Stephens, J. W. W., Blacklock, B., Macfie, J. W. S., Cooper, C. F., and Carter, H. F. Studies in the Treatment of Malaria, 91 113, 149, 165, 173, 283, 309, 331, 359, 417, 421, 425

INDEX OF SPECIES AND VARIETIES NEW TO SCIENCE

PAGE

Cylicostomum longibursatum 400
„ *minutum* 405
„ *nassatum*, Looss, var. *parvum* 411

PAGE

Spirochaeta intestinalis 79





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OBSERVATIONS ON MALARIA IN THE GOLD COAST COLONY, WEST AFRICA

BY

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AND

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WEST AFRICAN MEDICAL STAFF

(*Received for publication* 28 *December*, 1916)

PLATES I-II

INTRODUCTION

The Gold Coast shares with the rest of West Africa the unenviable reputation of being an unhealthy country, and although in recent years great improvement has been observed in the health of European officials, the latest (1914) return shows that the percentage of deaths to the average number resident was 1·70, a figure which must be considered high when it is remembered that it refers to individuals of an age with which death deals sparingly in Europe, who are moreover repeatedly subjected to searching medical examination.

From a purely medical point of view the reason for this is somewhat obscure, as many of the most serious diseases of other tropical countries are either unknown or are but rarely encountered in West Africa. Cholera, for example, has never yet broken out on the Gold Coast, and plague, although it has visited the colony, is not an established disease; kala-azar and other *Leishmania* infections, undulant fever, and tick fever are very rarely seen; typhoid fever is uncommon, and the trypanosomiasis that does occur is of the relatively mild type due to infections with *T. gambiense*. Dysentery, indeed, is prevalent, but it is mainly amoebic and is now-a-days fortunately amenable to treatment, and yellow fever and cerebrospinal meningitis periodically occur in fatal outbreaks.

Malaria, however, is exceedingly common, and is mostly of the sub-tertian variety. Official statistics are of little value in estimating

the incidence of this disease, because it is, as a rule, only the graver attacks that come to the notice of Medical Officers, the Europeans treating with quinine, and the natives ignoring, the milder manifestations; but, so far as they go, the annual returns for the four years 1911—1914 show that 10 per cent. of the admissions to Government hospitals, and 5 per cent. of the total attendances at Government dispensaries were attributed to malaria. In 1914, of a total of 499 on the sick list out of an average number of 646 resident European officials, malaria accounted for 180 and 'pyrexia' for 57. Amongst the natives other diseases such as pneumonia, tuberculosis, and ankylostomiasis are of great importance, but so far as the Europeans are concerned malaria dominates the field to such an extent that it must be concluded that the unhealthiness of the country is largely due to its prevalence.

THE RELATIVE FREQUENCY OF OCCURRENCE OF THE DIFFERENT MALARIA PARASITES

During the last three and a half years (1913 to June, 1916) a careful record has been kept of the results of the blood examinations made at the Accra laboratory, and some idea of the prevalence of the different malaria parasites in this district may be gathered from these figures (see Table I). Malaria parasites were found in 250

TABLE I.—The incidence of the malaria parasites in the blood of European and Native patients examined at the Accra laboratory.

	Europeans			Natives		
	Malignant tertian	Simple tertian	Quartan	Malignant tertian	Simple tertian	Quartan
1913	27	2	—	36	2	5
1914	42	2	3	18	1	1
1915	18	—	—	40	5	5
1916, Jan. to June ...	14	—	—	24	1	4
Totals ...	101	4	3	118	9	15
Percentages ...	40·4	1·6	1·2	47·2	3·6	6·0

cases, namely in 108 Europeans and 142 natives, and of these 219 (87·6 per cent.) showed sub-tertian parasites, 13 (5·2 per cent.) benign tertian, and 18 (7·2 per cent.) quartan. In both European and native patients sub-tertian parasites were by far the commonest, but in the former benign tertian and quartan parasites were relatively rarer than in the latter. The fact that all Europeans were adults but a considerable number of the natives young children, may account for this, as it has been recorded by Statham (1915) that at Freetown, Sierra Leone, benign tertian and quartan infections were very rare in adults, but amounted to as much as 20 per cent. in children.

The Investigators' Reports published by the Yellow Fever Commission (West Africa) furnish some interesting and at the same time puzzling statistics as to the occurrence of malaria parasites (see Table II). The Reports by the investigators in the Gold Coast

TABLE II.—The prevalence of the malaria parasites, according to various observers, in Sierra Leone and the Gold Coast.

Locality	Observers	Number of cases	Malaria parasites in percentages		
			Sub-tertian	Benign tertian	Quartan
Freetown, Sierra Leone	Statham	676	98·5	?	?
Freetown, Sierra Leone	Butler	201	95·0	0·5	4·5
Freetown, Sierra Leone	Dalziel and Johnson	221	77·7	0·4	21·9
Sekondi, Gold Coast ...	Coghill and Hänschell	259	56·8	18·0	25·2
Sekondi, Gold Coast ...	Coghill and Hänschell	211	72·5	11·1	16·4
Accra, Gold Coast ...	Laboratory records ...	250	87·6	5·2	7·2

(Coghill and Hänschell) show that at Sekondi from 1st May to 30th September, 1913, sub-tertian parasites were found in 189 persons (56·8 per cent.), benign tertian in 60 (18·0 per cent.), and quartan in 84 (25·2 per cent.), and that from 1st October, 1913, to 30th April, 1914, sub-tertian parasites were found in 177 (72·5 per cent.), benign tertian in 27 (11·1 per cent.), and quartan in 40 (16·4 per cent.).

At Freetown, Sierra Leone, Butler in 201 cases examined between 1st May and 14th September, 1913, found sub-tertian parasites in 191 (95 per cent.), benign tertian in 1 (0·5 per cent.), and quartan in 9 (4·5 per cent.); but from September, 1913, to March, 1914, Dalziel and Johnson found sub-tertian parasites in 174 (77·7 per cent.), benign tertian in 1 (0·4 per cent.), and quartan in 49 (21·9 per cent.). The five investigators quoted all agree in attributing the majority of infections to the sub-tertian parasite, but they differ considerably in the percentages they assign to the benign tertian and quartan organisms; and whereas at Sekondi the two latter types were found more frequently during the period May to September, at Freetown they were commoner from September to March. There may, of course, have been a natural explanation of these apparent discrepancies, but as crescents are notoriously difficult to find in the peripheral blood in West Africa, a fact which was remarked also by these investigators, most of the identifications must have been made from ring forms alone, and it is more probable that they depend on the personal factor. The same explanation may perhaps account for the fact that Connal (1912) assigned 35·4 per cent. of the malaria cases he examined at Accra to the quartan type, an observation which has not been confirmed by any subsequent worker.

Rogers (1910) has collected some data regarding the prevalence of different varieties of malaria in India, which are of interest in this connexion. In Calcutta sub-tertian cases constituted 54·2, benign tertian 38·6, and quartan 7·2 per cent. of 539 malaria cases; in Bombay the percentages were respectively 46, 52·6, and 1·4; in Madras sub-tertian infections were commonest and quartan rarest; and in Bareilly, in the United Provinces of Agra and Oudh, benign tertians accounted for over 90 per cent. of the cases. So far as these figures go, they seem to prove that sub-tertian infections are very much more common in West Africa than they are in India.

The rarity of crescents in sub-tertian infections in West Africa has been commented on by practically every investigator who has recorded observations on malaria in this region. Statham (1915), as the result of a careful study of 676 adult cases of malaria at Freetown, Sierra Leone, all but ten of which were due to the sub-tertian parasite, has suggested that 'the sub-tertian parasite of

West Africa is somewhat different from that found in Asia.' The reasons he gives in support of this view are (1) that the gametes are more rarely found, (2) the great difficulty occasionally met with in finding parasites in cases which are clinically typical, and (3) the greater amenability of the disease to quinine treatment. In a section which follows, attention is drawn to certain morphological peculiarities which, if they prove to be less commonly met with in other countries, may lend support to Statham's suggestion.

THE SEASONAL INCIDENCE OF MALARIA AT ACCRA

There are not yet sufficient accurate data on which to base a study of the seasonal incidence of malaria in the different parts of the Gold Coast; but during the last two years, since the laboratory was started on its present footing, it has been possible for the Medical Officers at Accra to have the blood of any patient suspected of suffering from malaria examined for parasites, and the results should give some idea of the prevalence of this disease in this town during the different seasons of the year. The total number of cases examined has been small, and it must be admitted that not all the 'clinical' malarias have been investigated, but such as they are the results are suggestive and should perhaps be recorded. It is possible, however, that some modification may have to be made in our views when further statistics are available.

From September, 1914, to August, 1916, two complete years, malaria parasites were found in 195 cases of fever at Accra, and of these 181 were sub-tertians, 8 benign tertians, and 6 quartans (see Table III). The number of benign tertian and quartan infections were too few to indicate any seasonal variation, but there was a great difference in the monthly distribution of the sub-tertians. Cases of this form of malaria occurred throughout the year, but in July the number suddenly increased, reached a maximum in August, and in September fell rapidly towards the mean level. The actual figures, set forth in Table III, show that 62 per cent. of the sub-tertian cases occurred during the third quarter of the year. This very remarkable seasonal variation appeared to be directly dependent on the rainfall; for although Accra is a relatively dry town, what

rain there is falls mostly in June, and, as will be seen by reference to Chart I, the curve formed by plotting the cases of malaria followed closely the curve of the rainfall during the two years under consideration.

It is of some interest to compare the distribution of the cases of sub-tertian malaria at Accra with those for different parts of India summarised by Rogers (1910). In Calcutta sub-tertian infections accounted for 54·2 per cent. of 539 malaria cases, but no less than 61 per cent. of them occurred during the last three months of the year. That is, the season of prevalence of malignant infections was

TABLE III.—The prevalence of malaria at Accra during the two years, September, 1914 to August, 1916.

Type of malaria	Month											
	January	February	March	April	May	June	July	August	September	October	November	December
Sub-tertian	3	6	10	4	12	12	39	57	17	4	9	8
Benign tertian ...	1	—	1	—	1	1	1	2	—	1	—	—
Quartan	2	—	—	—	—	1	—	2	—	—	—	1
Totals	6	6	11	4	13	14	40	61	17	5	9	9
Rainfall in inches ...	—	0·45	3·93	3·65	7·83	29·89	5·47	1·67	0·56	0·82	2·42	0·07

relatively short and occurred during the drying up at the end of the south-west monsoon. In Bombay, where the temperature is more uniform, sub-tertian cases occurred more regularly throughout the year, and accounted for 46 per cent. of the infections, and in Madras the seasonal incidence was said to be similar. Both in India and at Accra in the Gold Coast the season of greatest prevalence of sub-tertian infections occurs, as might be expected, during the drying up period following the rains; but in Bombay and Madras, as at Accra, this type of malaria prevails all the year round, a fact that should probably be correlated with the uniform temperature.

Not every case of malaria is a fresh infection, but it is nevertheless probable that the majority of the cases occurring during the third quarter of the year at Accra were of this nature. There was no

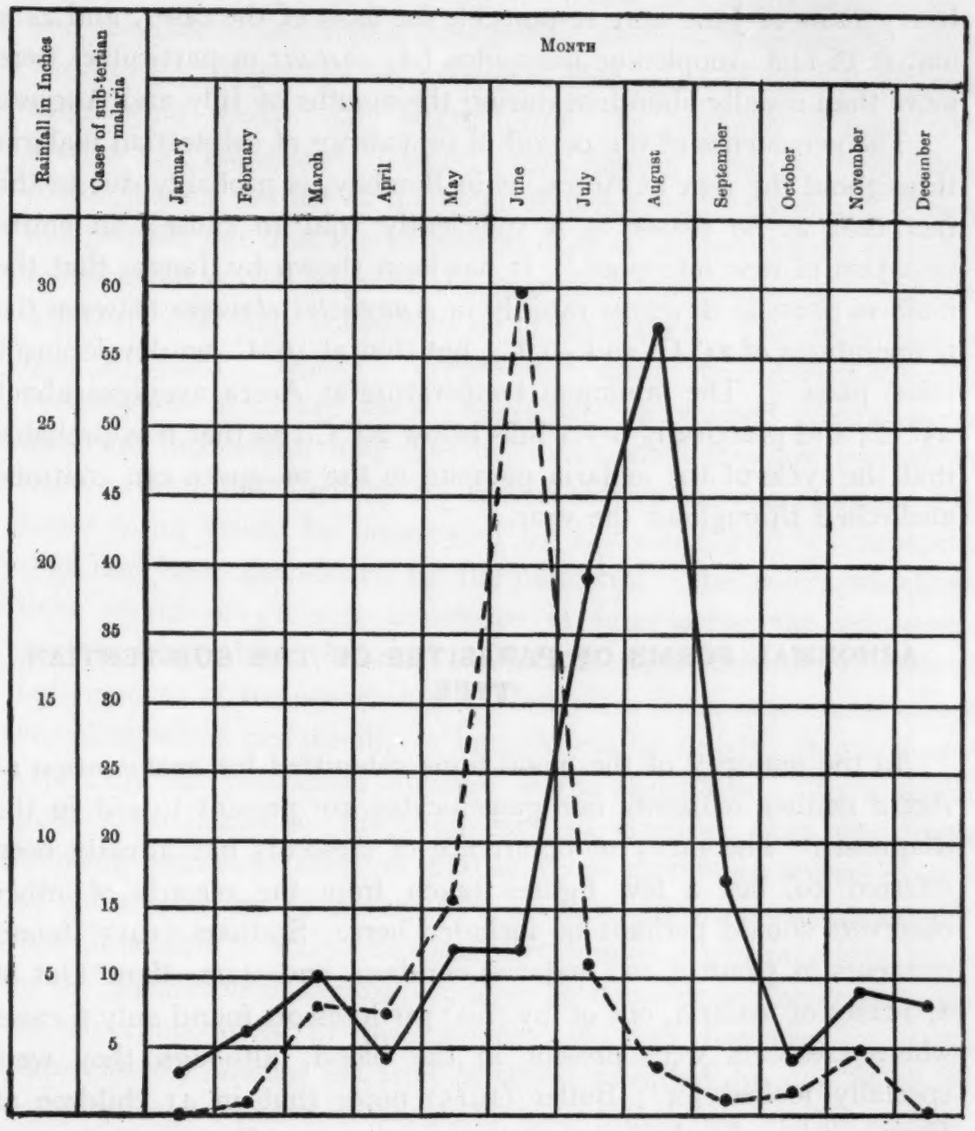


CHART I.

The rainfall (●- - - -●- - -) and the cases of sub-tertian malaria (●——●—) occurring at Accra during the two years, September, 1914 to August, 1916.

coincident fall of temperature such as might have induced a number of relapses, but it is possible that there may be a seasonal influence which determines relapses and that in the case of sub-tertian malaria

at Accra this comes into force in the third quarter of the year. The manner in which the curve of malaria cases followed the curve of the rainfall, as well as the interval shown between the curves, suggests, however, that the mosquitos bred in the pools formed by the heavy rains of June were responsible for most of the cases, and as a matter of fact Anopheline mosquitos (*A. costalis* in particular) were more than usually abundant during the months of July and August.

The persistence of the period of prevalence of sub-tertian malaria throughout the year at Accra, as in Bombay, is probably due to the fact that at no season is it sufficiently cold to cause 'an entire cessation of new infections.' It has been shown by Jansco that the malaria parasite develops rapidly in *Anopheles claviger* between the temperatures of 24° C. and 30° C., but that at 16° C. no development takes place. The minimum temperature at Accra averages about 23° C., and practically never falls below 20° C., so that it is probable that the cycle of the malaria parasite in the mosquito can continue unchecked throughout the year.

ABNORMAL FORMS OF PARASITES OF THE SUB-TERTIAN TYPE

In the majority of the blood films submitted for examination at Accra neither schizonts nor gametocytes are present to aid in the diagnosis. The rarity of occurrence of crescents has already been referred to, but a few figures taken from the records of other observers should perhaps be included here. Statham (1915) found crescents in 5 out of 100 malarial children, and states that 'Out of 176 cases of malaria, one of my (his) predecessors found only 6 cases where crescents were present in the blood, although they were specially looked for'; Butler (1915) notes that in 41 children at Freetown found to harbour sub-tertian parasites 'Crescents appeared to be conspicuous by their absence, for they were only found in one of the films'; and Coghill and Hänschell (1915) found gametocytes in only 8 out of 189, and 17 out of 177 sub-tertian infections; that is, in 683 cases of sub-tertian malaria examined by these investigators crescents were found in only 37, or 5·4 per cent. Schizonts are probably even rarer, and have only been detected once during the

last two years in the blood films examined by one of us (J. W. S. M.) at Accra.

The identification of the parasites has therefore to be made in the great majority of cases from the characters of the trophozoites, and this has necessitated a most careful study of their morphological variations. Rogers (1910) considers that when a number of small rings occur without any larger forms 'the infection is practically always a malignant tertian, this being, indeed, the most distinguishing feature of the blood in this form of malaria.' Powell has pointed out that the chromatin dots of the sub-tertian parasites are situated at the edge of the ring and tend to project slightly, a feature that may sometimes be of assistance. The occurrence of the so-called 'brassy' corpuscles, and the darker-coloured erythrocytes that are their counterpart in stained preparations, although inconstant, may also help, but when they are present we have noticed that they by no means always show parasites. The latter forms would be interpreted by Lawson (1916) as corpuscles that had been abandoned by the parasites. The points which we have found of greatest assistance in recognising the sub-tertian parasites have been the small size of the organisms, the absence of enlargement of the enveloping erythrocytes, the characteristic coarse stippling which can usually be brought out by suitable staining, and especially the precocious division of the chromatin. In a recent publication Lawson (1916) appears to consider that the forms showing precocious division, that is, those containing several masses of chromatin, are in reality two or more parasites superimposed. We are unable to accept this interpretation as a general explanation of the phenomenon, because we have been unable to detect any sign of superimposition in the majority of such parasites. In an exceptionally intense malarial infection recorded by one of us in 1915 a large number of parasites were found free in the plasma, some of which contained more than one chromatin mass, but none showed partially superimposed parasites. If, as Lawson believes, these forms are due to 'multiple infection of corpuscular mounds,' one would hardly expect to find them free in the blood.

The morphology of what we refer to as the sub-tertian parasite is extremely variable, and the 'normal' ring type, although it is certainly the commonest form both in severe and mild cases, is very

frequently departed from. In a few cases parasites without chromatin occurred (Pl. II, figs. 41 and 42), and one such infection was recorded by one of us in the Annual Report of the Accra Laboratory for 1914.

Stephens (1914) recently described what he considered to be a new malaria parasite of man, for which he proposed the name *Plasmodium tenue*, characterised by its extremely amoeboid form and the abundance and irregularity of its nuclear chromatin. This organism was discovered in a blood film taken from a native child in India. The same patient was examined on several subsequent occasions and was found to be infected with quartan parasites, and once 'a single pigmented (presumably simple tertian) parasite was found.'

Considerable discussion has followed the publication of Stephens' paper. Craig (1914) 'is satisfied that *Plasmodium tenue* is an atypical form of *Plasmodium vivax*', but Balfour and Wenyon (1914) consider that Stephens 'has not produced any evidence to prove that he was not dealing with an amoeboid sub-tertian parasite'. The latter observers figured parasites closely resembling *P. tenue* from three cases from the Soudan, West Africa, and the Persian Gulf respectively, in two of which crescents also were found.

At Accra we have frequently found malaria parasites which we believe to be indistinguishable from *P. tenue* (Pl. II, figs. 1-8). In 103 consecutive cases specially examined for these forms they were found in 18. Sometimes all the parasites in the blood films were of this type, but usually only a larger or smaller proportion of them, and in such cases the other forms were with one exception typical sub-tertian rings. The abundance and irregularity of the nuclear matter appeared often to be due to two or more parasites infecting a single red cell and being more or less completely interlaced (Pl. II, fig. 5). In several cases crescents were found, and in many others a coarse stippling was demonstrated by suitable staining. For these reasons we concluded that it was impossible to differentiate between *P. tenue* infections and sub-tertian infections such as occur in West Africa, and accordingly in the Annual Report of the Accra Laboratory for the year 1915 both types were classed under the heading *P. falciparum*. Of the sub-tertian infections thus defined, over 20 per cent. showed *P. tenue* forms.

The one case noted above as being exceptional showed a mixed

infection with *P. tenue* and quartan parasites. The amoeboid parasites occurred in corpuscles which showed a characteristic coarse stippling, and for this reason we incline to the view that they were distinct from the quartan organisms, which in this case had not produced any stippling.

Benign tertian malaria is uncommon at Accra, and could not possibly account for the large number of cases in which *P. tenue* forms were found. One native child with a heavy infection with *P. vivax* was examined in whom the parasites showed an extreme degree of amoeboid irregularity. It was impossible, however, to confound these forms with *P. tenue* owing to the large size of the organism, the distension of the corpuscles, and the characteristic stippling.

Stephens (1914) has described yet another form of malaria parasite, for which, however, he has proposed no name. This organism was found in the blood of a native child in the Gold Coast, and the most characteristic forms were 'chromatin particles or strands without any protoplasm.' In the same blood film normal quartan rings were found, and all stages could be traced between these and the remarkable forms peculiar to this infection. We have met with this form of malaria parasite on four occasions at Accra (Pl. II, figs. 10-19). We have not found it associated with the quartan organism, however, but we have observed a coarse stippling of the infected erythrocytes which led us to suspect that this form should also be referred to the sub-tertian type.

Stephens states that three views are possible as to the nature of these forms, namely (1) that they are new species of parasite, (2) that they are degenerative, and (3) that they are artificial; and he considers that the fact that forms are found in which chromatin alone occurs is in favour of one of the latter two views. The fact recorded by one of us (1914) that in blackwater fever parasites without visible cytoplasm may occur is also perhaps in favour of these views.

The frequency with which we have found malaria parasites resembling *P. tenue* associated with typical sub-tertian parasites and sometimes crescents, or enclosed in red corpuscles exhibiting the characteristic coarse stippling (Stephens' and Christophers' dots),*

* This coarse stippling was first described by Stephens and Christophers (1900), and subsequently by Maurer (1902). It is therefore incorrect to call it 'Maurer's dots' as is sometimes done.

convinces us that these forms are in many cases at any rate abnormal forms of the sub-tertian organism; and for similar reasons we believe that the second new form described by Stephens must be interpreted in the same manner. The cases examined by Stephens appeared to be associated with quartan parasites, and it is possible that these abnormal forms may also occur in infections with this organism, but on this point we have no evidence from our own experience.

We have already stated that we have found *P. tenue* forms in over 20 per cent. of the cases diagnosed by us as sub-tertian malaria. It cannot but appear a remarkable fact that these forms should not have been repeatedly noted if they occur with similar frequency elsewhere. In West Africa it is only comparatively recently that microscopical examinations have become possible as a matter of routine, and it is not surprising that the occurrence of such forms has not previously been recorded; but this cannot be said of other tropical countries. It has been suggested that the sub-tertian malaria in West Africa is different from the Asiatic variety, a theory that would receive some support if it proves to be the fact that *P. tenue* forms are more frequently met with in this part of the world.

If, then, *P. tenue* and the second form of malaria parasite described by Stephens are in reality abnormal forms of the sub-tertian and perhaps the quartan parasites, on what does their occurrence depend? Balfour and Wenyon state that such 'amoeboid forms . . . are occasionally found, more especially in the late stages of those cases which have very large infections and which often terminate fatally', but it must be admitted that in exceptionally intense infections and in fatal cases these forms are not always found. The same authors suggest 'some mechanical explanation' without, however, clearly defining it. Lawson (1916) has recently supported the view that *P. tenue* forms are due to distortion by technique, and has denied that they represent either a new species or an amoeboid parasite. Some of the forms she figures as examples of *P. tenue* which particularly favour this view can hardly be considered as identical with those described by Stephens, and it is difficult to believe that the more amoeboid forms we have repeatedly observed could have been produced in the manner suggested. But even if it were the fact that these forms were produced by distortion the

difficulty is only put back one step, since it still remains to be explained why it is that these forms are found in some patients but not in others, when the same technique is employed in making the films in every case.

It has been suggested that *P. tenue* forms might result from spreading the blood on a slide which had been raised to an unusually high temperature by exposure to sunshine or some such condition. All the blood films examined by us were taken on slides at the ordinary atmospheric temperature, so that this explanation could not have been the correct one. By actual experiment, spreading films from a single drop of blood on slides at various temperatures we failed to produce, in a case of benign tertian malaria, highly amoeboid forms such as we had seen in a previous patient. When the slide on which the blood was spread was sufficiently hot most of the red corpuscles broke up into small globules, but those that were parasitised remained intact, and the parasites themselves were just as compact as those in the control slides (Pl. II, figs. 20 and 21). An abnormally high degree of fever had also to be abandoned as an explanation, as many of the patients in whom abnormal parasites were most numerous had little or no fever.

Most of the patients in whom we have found the abnormal forms of malaria parasites have been native children with greatly enlarged spleens and a history of repeated attacks of 'fever.' Out of 28 cases examined by us up to the present, 16 (equal to 57·1 per cent.) were children under two years of age, and only 8 (equal to 28·6 per cent.) were adults. The children were usually greatly debilitated and highly anaemic, and nucleated red corpuscles (normoblasts) were abnormally numerous in some cases. The adults in whom abnormal parasites were found were also anaemic and debilitated, and the constitutional disturbances associated with an attack of malaria were unusually severe. It appeared to us that the occurrence of the atypical parasites was definitely associated with the debilitated condition of the patient. As a rule, quinine treatment immediately banished the malaria parasites from the peripheral blood, but in two cases they persisted for a day or two. The one case, a child aged $3\frac{1}{2}$ years, when first examined showed *P. tenue* forms and crescents in the blood, but two days later the atypical parasites had all vanished and only crescents and typical sub-tertian parasites in

corpuscles showing coarse stippling were found. The second case was an adult European man in a very critical condition and with a heavy malaria infection. When first seen *P. tenue* forms were numerous, but there were also some typical sub-tertian rings and crescents. Three days later, after vigorous treatment with quinine, a few crescents and a few normal sub-tertian rings were all that could be found.

The suggestion of Balfour and Wenyon that the presence of *P. tenue* forms implies some diminished resistance on the part of the patient is supported by these observations, the quinine assisting the patients in combating the infection. But as in fatal cases of malaria only typical parasites may be found, it must be concluded that the anaemia is an important factor, either through some chemical change in the plasma or a physical action on the corpuscles.

THE ASSOCIATION OF MALARIA AND OEDEMA

Although it is generally admitted that nephritis may occur in malaria infections, but little attention appears to have been paid to this complication, and great differences of opinion exist as to its frequency. Castellani and Chalmers (1913) state briefly that 'Nephritis may be found in tertian and sub-tertian fevers, being directly due to the irritation of the kidney by the malarial toxins'; Rogers (1910) considers that the urine 'very rarely shows albumen or other marked changes in uncomplicated cases', but Osler (1914) records that a moderate albuminuria is of frequent occurrence, having been observed in 46·4 per cent. of the cases treated in his wards, that acute nephritis occurred in over 4·5 per cent. of his cases of aestivo-autumnal malaria, and that chronic nephritis occasionally follows long-continued or frequently repeated infections; and according to Henson (1913) nephritis is 'a very common complication,' and he attributes to malaria the high mortality rate from this affection, which he affirms is 'so common in all tropical and sub-tropical countries.'*

So far as we are able to ascertain, the occurrence of nephritis in

* For further references to the occurrence of albuminuria in malaria, see the Fourth and Final Report of the Yellow Fever Commission (West Africa), which has been issued since the above was written.

malaria has most frequently been in imperfectly treated cases, or in patients who have suffered from repeated attacks, and it has more often been associated with sub-tertian infections than with either benign tertian or quartan. Acute nephritis in the active stages of malarial infections appears to be relatively seldom recorded, and it may therefore be of interest to describe briefly a series of cases observed by us which suggested that in the Gold Coast the condition may be commoner than is generally supposed.

Between the 17th February and the 30th August, 1916, nine native children brought for treatment to the dispensaries at Koforidua and Nsawam showed oedema of the face, hands and feet, and in three of them that were in a more advanced condition ascites was also present. The children were all under ten years of age, the youngest being about five and the oldest nine. Seven were boys and two girls. In some of the cases a history of gradual onset of the swelling following upon repeated attacks of fever was obtained from the parents or persons in charge of the patients.

The general appearance of the patients closely resembled that of a case of acute nephritis, indeed the first two were looked upon and treated as cases of this disease, and it was only after this treatment had been carried on for some time with but little improvement that quinine was tried in increased doses, with the result that the oedema began to resolve rapidly.

It is difficult in dispensary practice to induce patients, especially those coming in from out-lying villages, to take the trouble to bring with them specimens of urine or faeces for examination, so that the urine was examined in five cases only and the faeces in four. None of the specimens of urine was 'smoky,' but no microscopical examination could be made for blood or tube-casts; all five, however, contained albumen. Three of the four specimens of faeces contained ova of *Ascaris lumbricoides*, but no hookworm ova were seen, and no ova of other helminths. None of the patients showed any involvement of the peripheral nerves. Two of them had slight fever when they attended for the first time, namely, 99·6° F. and 100·8° F. respectively.

Dried films of blood were made from all the cases, and were stained by Romanowsky's stain. Quartan parasites were found in every case, both ring forms, half-grown forms occasionally, and

sporulating forms; but in no case were they numerous. In one case there was a double infection with quartan and sub-tertian parasites (crescents). In these films the pigment appeared to be more finely divided and of a browner colour than is usually seen in *Plasmodium malariae*.

As already stated, the first two patients were considered to be suffering from acute nephritis, and they were therefore ordered a milk diet, given saline purges and a diuretic mixture together with two grains of quinine daily. This treatment was found to produce but little improvement, and accordingly the quinine was increased to nine grains daily, with the result that the oedema began to resolve at once and had practically disappeared within ten days of the alteration in dosage. After this experience the other cases, when they presented themselves, were given a saline purge and from six to nine grains of quinine daily, the diuretic being dispensed with. It is no easy matter to keep in touch with dispensary patients living at a distance, but most of these cases were seen three or four times at intervals of four days, and they invariably improved under the treatment with quinine alone. Once the improvement was marked they ceased to attend, contenting themselves with sending in for medicine at intervals. It is thus not possible to state whether any of them relapsed or not, but two cases resident in Koforidua which could be kept under observation had not relapsed four months after the disappearance of the oedema. The dosage of quinine in these two cases was gradually reduced to two grains daily.

Subsequently another case, probably of the same nature as those described above, was met with at Accra. The patient was a little girl about four years of age who was brought to the hospital because she had been 'swelling' for some weeks. The general appearance of the child is sufficiently shown in the photograph (Pl. I), which indicates clearly the oedema of the face, the legs and the hands, and the enormous distension of the abdomen which was due to free fluid. The blood was pale and watery, and in the red cells numerous malaria parasites were found. The urine was pale yellow in colour, acid in reaction, with a specific gravity of only 1005, and contained a fair amount of albumen and a number of hyaline casts. The faeces did not contain anything of pathological importance, and no ova of helminths could be found. The child was treated

with quinine, 2 grs. thrice daily, and rapidly began to improve; but unfortunately about a week later the parents insisted on taking her away, so that the final result was not observed.

This child had a mixed malaria infection. There were in the blood a few crescents and a few small sub-tertian rings, but the majority of the parasites were of an unusual type (Pl. II, figs. 22-35). They were compact organisms which appeared not to have been actively amoeboid; they were rich in chromatin, which in pre-sporulating forms was divided into six or eight pieces; they showed pigment of a brown colour at an early stage of their development; and the parasitised erythrocytes were not enlarged, but were heavily stippled with Schuffner's granules and occasionally with coarser particles more closely resembling Stephens' and Christophers' dots. The parasites themselves resembled quartan organisms more nearly than either of the other two types, and this is perhaps what they were, because on a subsequent occasion when the blood was examined similar forms were found, but without the stippling of the erythrocytes. It has been pointed out by Lawson that both Schuffner's granules and coarse stippling may be found in quartan infections, and, admitting this, there was no great reason to hesitate as to the identification of these parasites, although their general appearance was not quite like that of *P. malariae*.

The organism appeared to us to resemble *P. malariae* and to differ from *P. vivax* in the following respects:—The young trophozoites were smaller than those of *P. vivax* and their pseudopodia were not long, the pigment appeared early and was often arranged peripherally, the schizonts were about the size of red corpuscles, the merozoites numbered about eight, and the infected erythrocytes were about normal in size and colour. Unlike *P. malariae* the pigment was rather fine and brown, and Schuffner's granules were observed on the first day the blood was examined.

Ahmed Emin (1914) has described a malaria parasite, for which he proposed the name *Plasmodium vivax* var. *minuta*, which in many respects closely resembled that described above. It is not quite easy to distinguish in his figures chromatin from cytoplasm, and pigment granules from Schuffner's dots, but the young ring forms, five of which were often found in a single erythrocyte, resembled young trophozoites of *P. falciparum*. The youngest

forms of the parasite we have described were more compact and coarser structures, and were unlike the delicate rings described by Ahmed Emin. It is possible that had our case been examined at some other stage such forms might have been found, although it is not probable, or perhaps some of Ahmed Emin's patients may have had a concurrent infection with *P. falciparum*, and admitting this, the two parasites (*P. vivax* var. *minuta* and the one found at Accra) would appear to have been indistinguishable. No mention is made by Ahmed Emin of any association with oedema in his cases.

The parasites found in the other cases of malaria associated with oedema have been referred to above as quartan organisms (*P. malariae*). The organisms found after the first day in the case just described, that is when no stippling of the red corpuscles was observed, were more like *P. malariae* than either *P. vivax* or *P. falciparum*. The occurrence of the stippling seemed to be uncertain, so that little reliance can be placed on it. It is possible, therefore, that the parasite in all these cases may have been the same species.

It may be of interest in connexion with this action of quinine in these cases of oedema which were in all probability of malarial origin, to give the history of a case showing extensive oedema with much albumen in the urine which appeared to react to adequate doses of this drug, although no malaria parasites were found in the blood.

Mrs. B., a native aged 20 years, a primipara, was seen for the first time on the 29th April 1916, when she stated that she was five and a half months' pregnant. Her condition was serious, the whole of her lower extremities were markedly oedematous, as were also her hands and face; she was unable to lie down in bed, she was dyspnoeic on the slightest exertion, her pulse rate was 120 per minute, and there was dullness on percussion over the bases of both lungs posteriorly. The urine was scanty in amount and loaded with albumen. The patient had had no fits, and her mental condition appeared quite clear.

Treatment was begun by giving saline purgatives and a diuretic mixture containing digitalis, but at the end of a week, the condition of the patient being but slightly improved, she was ordered five grains of quinine three times a day in addition. By the end of another week the patient had greatly improved, her orthopnoea had

gone, the quantity of urine passed had doubled, while the albumen was reduced to a mere trace. The diuretic mixture was then stopped, but the saline purges were continued regularly every second day together with the quinine in diminished quantity, ten grains a day. She continued to improve rapidly, the oedema disappeared, the urine lost all trace of albumen, she was able to get out and about, and eventually during the second week of August she was delivered without assistance of a living full-time child. This patient took ten grains of quinine daily for nearly three months.

It is open to doubt whether this case was due to malaria, as no parasites were found, but it was not until after the patient had had fifteen grains of quinine that the blood was examined.

Albuminuria is probably by no means an uncommon symptom in children suffering from malaria, but the interest of these cases lies in the occurrence of oedema, the association of the condition with quartan-like infections, and the rapid recovery following quinine treatment.

In a popular lecture delivered by Dr. Oswaldo Cruz, and subsequently published in the 'Brazil Medico' (1915), a peculiar type of malarial fever existing in the Amazon Valley was referred to which was characterised by a liability to oedema of the legs. This disease was said to have been studied by Chagas, and to have been due probably to a distinct variety of quartan parasite. We have been unable to trace the work of Chagas referred to by Dr. Cruz, and it is possible that it has not yet been published; but the association of oedema with a quartan organism in the Amazon Valley is of interest in view of the discovery of an apparently somewhat similar type of malaria in the Gold Coast. With this exception we have not been able to find any reference to a malaria of this type in the literature at our disposal.

THE OCCURRENCE OF ANAPLASMA-LIKE BODIES IN THE BLOOD OF PATIENTS SUFFERING FROM MALARIA

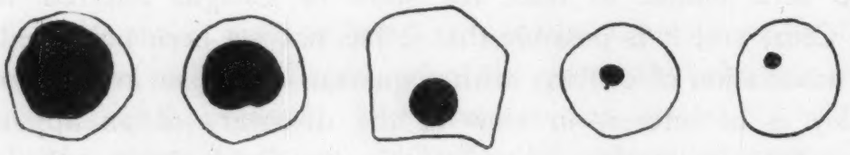
Anaplasma-like bodies are frequently met with in examining the blood of animals. At Accra, for example, we observed them without special search in 1914 in the blood of the following healthy animals: monkey, bat, donkey, hedgehog, cat, brown rat, black rat, pouched rat, mouse, guinea-pig, cattle, sheep, and goats, and

in pigs 'they were found, even in this casual way, in 33 per cent. of the animals examined.' They have also been found by other observers in a great many different hosts.

From time to time these bodies have been associated with various parasitic infections such as piroplasmosis, gall-sickness, trypanosomiasis, leishmaniasis, etc., and it has been shown that they can be produced by inoculations of phenylhydrazine and other substances. They are apparently most frequently found in young or anaemic animals, or in blood containing haemolytic substances, but there can be no doubt that they are very far from uncommon in perfectly healthy adult animals.

It is therefore improbable that these Anaplasma bodies are organismal, and although as Porter (1915) has pointed out, they 'are probably of diverse origin,' it is perhaps most likely that they are nuclear structures.

Gulland and Goodall (1912) state that the process by which normoblasts give rise to erythrocytes consists in the internal disintegration and absorption of the nucleus, the old view that the nucleus was extruded being now almost entirely given up. In the blood of a young specimen of the wild 'hare' of the Gold Coast recently examined by us, both Anaplasma bodies and normoblasts were abundant, and every stage could be traced between the two (see Text-figs. 1-5). In this case it could hardly be doubted that



1 2 3 4 5

the Anaplasmata were the remains of incompletely absorbed nuclei.

Anaplasma-like bodies are also common in human blood, and have been associated with infancy, leishmaniasis, anaemia, etc. Porter (1915) has recorded the occurrence of these bodies in 'the blood of a man returned from the tropics, who had perhaps previously had malaria.'

During the last two years we have repeatedly observed Anaplasma-like bodies in human blood at Accra. At first we paid but little attention to them, attributing their occurrence to anaemia,

but eventually our suspicions were aroused and a more careful study of their incidence was begun. It was then found that they were quite common in cases of malaria, and were often present in the blood of patients suffering from conditions diagnosed clinically as malaria and which responded readily to quinine treatment when no parasites could be found. Most of the latter cases were Europeans who took prophylactic doses of quinine with more or less regularity. They were also found in blood films in which crescents or pigmented leucocytes occurred, but no vegetative forms of malaria parasites.

Three possible explanations of the nature of these bodies occurred to us, namely:—

(1) They might be Anaplasmata—that is, incompletely absorbed nuclei.

(2) They might be imperfectly stained plasmodia.

(3) They might be forms of malaria parasites devoid of cytoplasm.

Anaemia is of course a common condition in the tropics, but the high degree usually associated with the occurrence of Anaplasmata is not so very frequently met with, and it seemed unlikely that the large number of cases in which these bodies were found could all have been anaemic. Some of the patients, as a matter of fact, were not notably anaemic at all. The bodies we found associated with malaria appeared, moreover, to differ slightly from those found in anaemia.

Anaplasmata are small, spherical, chromatin-staining bodies. They vary somewhat in size, according to Porter (1915), from 0·3μ to 2μ in diameter, but forms about 0·5μ in diameter are relatively numerous. They almost always are round, but may be oval; and their outline is generally smooth and sharp, very rarely irregular. By Romanowsky methods they stain intensely a deep chromatin colour, but do not show any characteristic structure. The bodies found by us (Pl. II, figs. 36-40) resembled exactly in size, shape, and staining reactions the round chromatin masses seen in a typical sub-tertian ring (*P. falciparum*). They appeared to be rather more uniform in size than Anaplasmata, and never reached the large sizes to which these bodies may attain. They also appeared to stain less deeply with Leishman's stain, as though their structure were less dense. The distinction, however, was not very marked, and the colour varied

of course with the intensity of the staining, and we were doubtful about it until we examined a case in which a considerable number of normoblasts were present. In the blood of this patient both Anaplasmata and the chromatin bodies associated with malaria were found, and on comparing them there was undoubtedly a difference.

Peculiar malaria parasites were observed in two cases of black-water fever by one of us (1914), in which in spite of intense staining the cytoplasm was all but invisible, being indicated only by a paler area or halo beside the chromatin particles. At the time it was suggested that this peculiarity might have been due to the abnormal condition of the blood (haemoglobinaemia) accompanying the haemoglobinuria.' Lawson (1916) has recently stated that in blood films malaria parasites may be found partially stained, for instance, with only the chromatin stained, and she explains this phenomenon as being due to fading, and affirms that it is 'usually possible to re-stain these specimens bringing out the faded cytoplasm.' Malaria parasites may therefore simulate Anaplasmata, and the bodies we have observed might be explained in this manner. We have already stated that they exactly resembled the chromatin particles of typical sub-tertian rings, but we do not believe that they were due to irregular staining because they appeared to us to differ from such forms, with which we are familiar; re-staining never resulted in revealing the presence of cytoplasm, and they were nearly always spherical and less variable in shape than the chromatin particles of ordinary malaria parasites. The specimens were always examined immediately after they had been stained, so that 'fading', in the ordinary meaning of that term, could not have taken place.

The third theory is that they might be malaria parasites devoid of cytoplasm, and this we believe to be the true explanation. Their close resemblance to the chromatin masses of malaria rings, their association with typical plasmodia in some cases, and their occurrence in patients suffering from an illness clinically resembling malaria, which was moreover amenable to treatment with quinine, support this view. They differed, however, from the forms described by Stephens in his Gold Coast case in which 'chromatin particles or strands without any protoplasm' occurred, in being rounded and in occurring singly in the erythrocytes.

If, then, they are of this nature, what is their significance? As

we have already stated, they were most frequently found in Europeans accustomed to take prophylactic doses of quinine or who had already taken quinine since their illness began. In such patients it is often extremely difficult or impossible to find parasites, although the physician attending them may be morally certain that they are suffering from malaria. We incline to the view that these Anaplasma-like bodies are due to the action of quinine in the blood, that is, that they are degenerative. In this connexion it is of interest to recall that in blackwater fever, which also eliminates the parasites from the blood, somewhat similar forms were found, but that in this case the degeneration did not appear to have proceeded so far.

The practical importance of the recognition of these forms is that it enables a diagnosis of malaria to be suggested in those cases, by no means uncommon, in which no typical parasites can be discovered.

ACCRA, *November*, 1916.

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EXPLANATION OF PLATES

PLATE I

A Case of Malaria associated with Oedema.



A CASE OF MALARIA ASSOCIATED WITH OEDEMA

C. Tinling & Co., Ltd., Imp.

PLATE II

Various forms of malaria parasites. × 2000.

Drawings by Miss Mabel Rhodes.

Figs. 1-8. Parasites resembling *P. tenue*.

Fig. 9. A sub-tertian parasite with a faint halo as the only indication of the presence of cytoplasm.

Figs. 10-19. Various forms of parasites with little or no visible cytoplasm.

Figs. 20-21. *P. vivax* in blood spread on a slide heated sufficiently to cause most of the red corpuscles to break up.

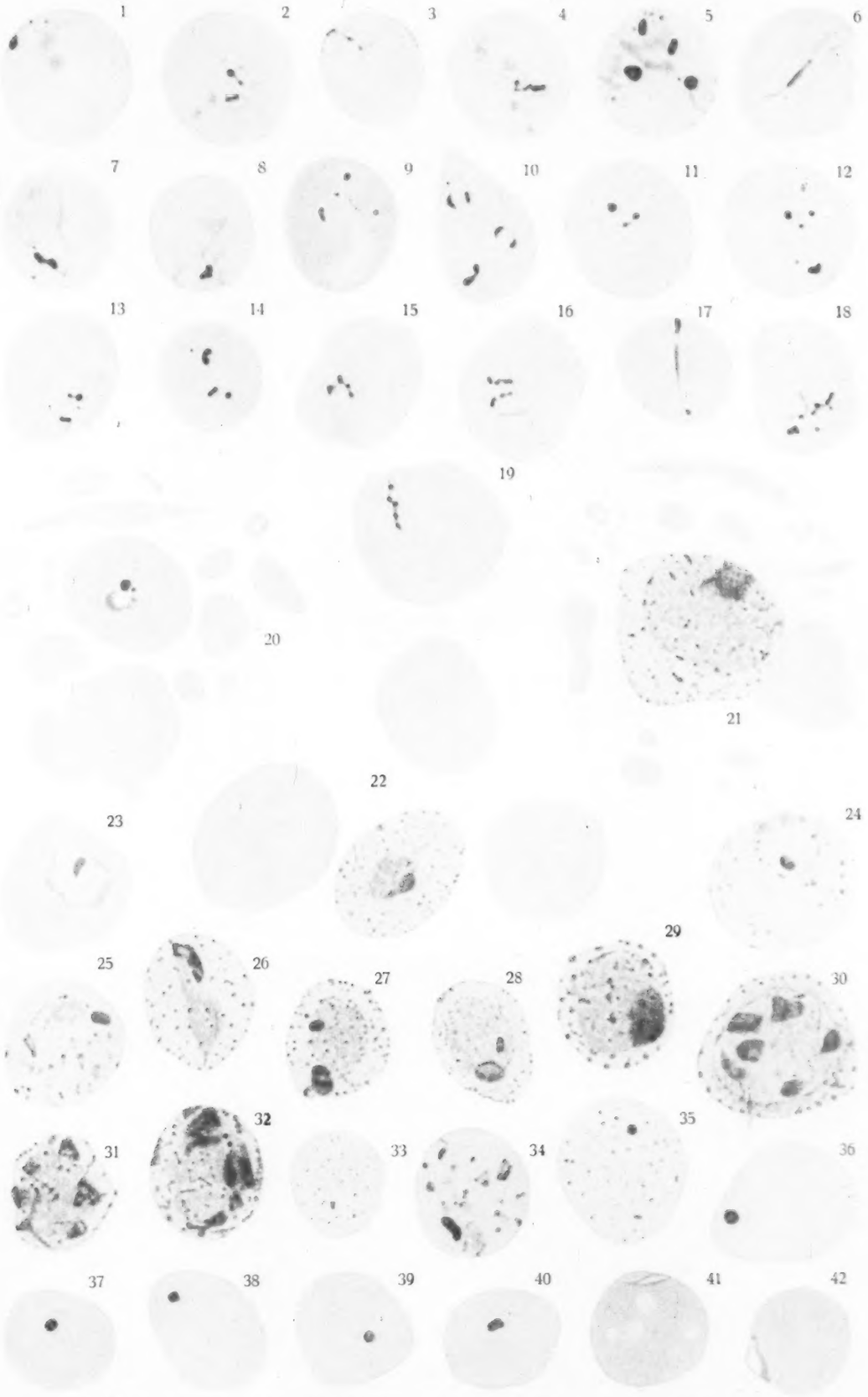
Figs. 22-35. Parasites of an unusual type from a case of malaria and oedema.

Fig. 22. A parasitised cell and three normal red corpuscles to show the relative sizes.

Figs. 33-35. Red corpuscles showing stippling but no parasites.

Figs. 36-40. Anaplasma-like bodies associated with malaria infections.

Figs. 41-42. Parasites (*P. falciparum*) devoid of chromatin.



MALARIA PARASITES FROM THE GOLD COAST.

PROTOZOOLOGICAL INVESTIGATION OF CASES OF DYSENTERY CONDUCTED AT THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

(SECOND REPORT)

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(*Received for publication* 5 *April*, 1917)

CONTENTS

	PAGE
INTRODUCTORY	27
PART I. PROTOZOAL FINDINGS IN 826 CASES OF DYSENTERY EXAMINED FROM OCTOBER 1ST TO DECEMBER 31ST, 1916	28
PART II. DISCUSSION OF THE COMBINED RESULTS FOR THE PERIODS MAY 1ST TO SEPTEMBER 30TH AND OCTOBER 1ST TO DECEMBER 31ST, 1916	32
PART III. STATISTICAL CONSIDERATION OF THE COMBINED RESULTS	48
PART IV. TWENTY CASES OF INFECTION WITH *E. histolytica* FROM THE FRENCH FRONT	59
PART V. TREATMENT OF *E. histolytica* INFECTIONS	63
REFERENCES	68

INTRODUCTORY

In our First Report (1917) we published the results of the examination of 910 cases of dysentery conducted at the Liverpool School of Tropical Medicine. That report closed at the end of September, 1916, and in Part I of the present contribution there are given the results of the examination of 826 cases that have come

under our observation during the months of October, November, and December, 1916. In our first report we made a few observations on certain points of interest which arose from a survey of the records, and we anticipated a fuller discussion on various questions connected with protozoological examinations when we had a greater number of facts at our disposal. For the purposes of this fuller discussion, which will be found in Parts II and III of the present report, we have combined the figures given in Part I with those of our first report, in order to obtain figures of as large a number of cases and examinations as possible. In Part III the combined figures are considered statistically and graphically, and certain conclusions are drawn which agree with and confirm the general conclusions arrived at in Part II. Since the period of our first report we have encountered a number of infections with *E. histolytica* apparently contracted in France, and the details of these cases are set forth in Part IV. Finally, the results of treatment of *E. histolytica* infections are presented in Part V.

PART I.—PROTOZOAL FINDINGS IN 826 CASES OF DYSENTERY EXAMINED FROM OCTOBER 1st TO DECEMBER 31st, 1916

MATERIAL

The first section of this report is devoted to a consideration of the protozoological work done during the last three months of 1916, on cases of dysentery or related intestinal disorders among troops invalided home. The information provided has been obtained from protozoological investigations conducted on 826 such cases. Microscopical examinations, for the detection of intestinal protozoa, of 3,930 samples of the stools of these patients were made at this laboratory. Nearly 900 cases were actually dealt with during this period, but the additional cases receive no further reference here, since they were included in our first report, and the subsequent examinations revealed no new facts of sufficient importance to warrant their reconsideration. The 826 cases reported on now are not wholly new, but include 23 of those in the previous report. These are incorporated here because each case showed one or more infections with certain organisms, after the first report was closed.

ANALYSIS OF RESULTS

The results obtained in regard to cases infected with any of the protozoal organisms concerned, and the incidence of infection of the various species* observed, are as follows:

Total number of cases examined 826

Number of cases having protozoal infections... 420 or 50·8%

In Table I an analysis of the 420 positive cases is given, and the incidence of infection shown.

TABLE I

	Number of cases infected	Percentage of total cases examined	Pure infections	Mixed infections
E. histolytica	94	11·4	26	68
E. coli	274	33·2	169	105
G. intestinalis	151	18·3	93	58
C. mesnili	36	4·4	14	22
T. intestinalis	7	0·8	1	6
Amoeba limax	1	—	—	—

The percentage of infection is, on the whole, somewhat higher than that given in our previous report.† There are appreciable increases in the percentages of cases infected with *E. histolytica*, *E. coli*, and *C. mesnili*, while the percentages of *G. intestinalis* and *T. intestinalis* cases are but slightly lower than before.

As denoted above, one patient frequently harboured two or more of these organisms. Some idea of the commoner associations noted may be gained from the list given below.

Double infections:

E. histolytica and *E. coli* in 36 cases.
E. histolytica and *G. intestinalis* in 8 cases.
E. histolytica and *T. intestinalis* in 3 cases.
E. coli and *G. intestinalis* in 36 cases.
E. coli and *C. mesnili* in 9 cases.
G. intestinalis and *C. mesnili* in 1 case.

* The organisms of importance are:—*Entamoeba histolytica* Schaudinn; *Entamoeba coli* (Lösch); *Giardia* (*Lamblia*) *intestinalis* (Lambl); *Chilomastix* (*Tetramitus*) *mesnili* (Wenyon); and *Trichomonas intestinalis*, Leukart.

† In that report the percentages of infections found were:—*E. histolytica* 10·3, *E. coli* 25·4, *G. intestinalis* 18·6, *C. mesnili* 2·7, *T. intestinalis* 1·2.

Triple infections:

E. histolytica, E. coli and *G. intestinalis* in 10 cases.

E. histolytica, E coli and *C. mesnili* in 8 cases.

E. histolytica, E. coli and *T. intestinalis* in 2 cases.

E. coli, G. intestinalis and *C. mesnili* in 2 cases.

E. coli, C. mesnili and *T. intestinalis* in 1 case.

Quadruple infection:

E. histolytica, E. coli, G. intestinalis and *C. mesnili* in 1 case.

DETAILS OF EXAMINATIONS MADE

An analysis of the total number of examinations made showed the following distribution:

826 cases had at least one examination each.
803 cases had at least two examinations each.
591 cases had at least three examinations each.
162 cases had at least four examinations each.
95 cases had at least five examinations each.
80 cases had at least six examinations each.

Table II gives the results obtained for the number of cases examined at each stage of the above analysis. The increase, in the number and percentage of both total and specific infections, at each additional examination is thus evident.

TABLE II

Number of cases examined	Examination	Total infected cases	Percentage of total cases examined	Cases infected with *E. histolytica*	Percentage of total cases examined	Cases infected with *E. coli*	Percentage of total cases examined	Cases infected with *G. intestinalis*	Percentage of total cases examined
		(*a*)		(*b*)		(*c*)		(*d*)	
826	First	266	32·2	46	5·6	151	18·3	90	10·9
803	Second	375	45·4	68	8·2	221	26·8	128	15·5
591	Third	413	50·0	89	10·8	248	30·0	144	17·4
162	Fourth	419	50·7	92	11·1	258	31·2	148	17·9
95	Fifth	419	50·7	93	11·3	261	31·6	149	18·0
80	Sixth	419	50·7	94	11·4	262	31·7	149	18·0
Ultimate results (*Vide* Table I)		420	50·8	94	11·4	274	33·2	151	18·3

The six new infections discovered at the fourth examination (column (*a*)) consisted of two of *E. coli*, three of *G. intestinalis*, and one of *C. mesnili*. In columns (*c*) and (*d*) the table shows that twelve infections of *E. coli* and two of *G. intestinalis* were discovered after the sixth examination. The *E. coli* infections were found in the following order: four at the seventh examination, two at the eighth, two at the twelfth, one at the fourteenth, one at the seventeenth, one at the thirtieth, and one at the forty-fifth. The two *G. intestinalis* infections were detected at the seventh and twelfth examinations.

The number of examinations made on the cases infected with *E. histolytica*, *E. coli*, and *G. intestinalis*, and the number of times each protozoon was found, are both set forth in Table III.

TABLE III

	Number of cases infected	Number of examinations made	Number of times found	Ratio of examinations to positive findings
E. histolytica	94	1708	410	4·2 : 1
E. coli	274	2013	666	3·0 : 1
G. intestinalis	151	759	352	2·1 : 1

The carriers of *E. histolytica* thus received a total number of 1,708 examinations, and the organism was found on 410 occasions; the ratio of examinations to positive findings is therefore 4·2 : 1, or, on an average, the parasite was detected once in every four examinations made. This ratio is lower than it would have been if the cases had received no treatment, owing to the large number of negative examinations induced by the treatment. It would have been still further reduced had closer supervision of the infected cases been possible. *E. coli* was found once in every three examinations, and *G. intestinalis* approximately once in two.

It is important to bear in mind that the figures given in Table III refer to all the cases we have examined, and since many cases received only a few examinations each, the ratios derived from the given data are open to the objection raised by Dobell (1917) in

his report to the Medical Research Committee. In order to obtain more reliable information regarding the frequency of positive and negative findings in any infection, e.g., *G. intestinalis*, we ought to consider cases which have been examined many times for some reason quite independent of their having this infection—for instance, because they were infected with *E. histolytica*. We should then get an entirely unbiassed and random selection of infected cases which would include those in which the infection showed itself infrequently as well as those in which it appeared frequently. This method is adopted in the discussion which follows in Part II, where the ratios of positive to negative findings receive further consideration.

ORGANISMS OTHER THAN PROTOZOA

Infections of a non-protozoal nature were sometimes observed. In twelve cases the eggs of the worm *Trichiuris trichiura* (L.) (= *Trichocephalus dispar*, Rudolphi) were found, and twelve patients were infected with the so-called 'I. bodies' of Wenyon.

SUMMARY

1. From October 1st to December 31st, 3,930 protozoological examinations were made on the stools of 826 cases of dysentery.

2. Of these, 420, or 50·8 per cent., were infected with protozoa.

3. *E. histolytica* was found in 94 cases (11·4 per cent.), *E. coli* in 274 cases (33·2 per cent.), *G. intestinalis* in 151 cases (18·3 per cent.), *C. mesnili* in 36 cases (4·4 per cent.), and *T. intestinalis* in 7 cases (0·8 per cent.).

4. The standard of non-infection for the majority (591) of the cases was three negative examinations.

PART II.—DISCUSSION OF THE COMBINED RESULTS FOR THE PERIODS MAY 1st TO SEPTEMBER 30th, AND OCTOBER 1st TO DECEMBER 31st, 1916

For the purposes of the following discussion we have combined the results of our first report with those presented in Part I of this report. The combined results have not been arrived at by simple addition of the figures given in the two reports, for it has been necessary to make allowance for a small number of instances in which the same case has been included in both reports. The first

report has been revised also with regard to the total number of examinations made, for additional examinations were made of some of the cases included in the first report after that report was closed. Thus the total number of examinations has been considerably increased. When these necessary corrections have been made, we obtain the following as the combined results:

Total number of cases examined 1,713
Total number of examinations made 8,894
Number of cases having protozoal infections... 818 or 47·8%

In the following table an analysis of the 818 positive cases is given:

TABLE IV

	Number of cases infected	Percentage of total cases examined	Pure infections	Mixed infections
E. histolytica	188	10·9	61	127
E. coli	505	29·4	307	198
G. intestinalis	317	18·5	193	124
C. mesnili	61	3·5	16	45
T. intestinalis	18	1	3	15

DETAILS OF EXAMINATIONS MADE

The total number of examinations performed was 8,894, which is an average of 5·2 examinations per case. This average is obtained from two distinct classes of cases, (1) the *E. histolytica* cases, on 188 of which approximately 4,000 examinations were conducted, an average of 22 examinations each; and (2) the cases in which *E. histolytica* was not found, on 1,525 of which about 5,000 examinations were made, an average of approximately three examinations each. The details of the actual examinations are as follows:

1,713 cases had at least one examination each.
1,560 cases had at least two examinations each.
1,093 cases had at least three examinations each.
459 cases had at least four examinations each.
301 cases had at least five examinations each.
236 cases had at least six examinations each.

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1,560 cases had at least two examinations each.
1,093 cases had at least three examinations each.
459 cases had at least four examinations each.
301 cases had at least five examinations each.
236 cases had at least six examinations each.

These figures give a clearer idea of the distribution of the examinations than is conveyed by expressing as an average the number conducted upon each case. The figures are important also because they have a direct bearing on the number of infected cases found. It has been our general experience that a number of cases recorded 'negative' in their first two or three examinations proved to be really 'positive' as a result of subsequent examination. We have also to bear in mind the method followed in making examinations. In ordinary routine work we have examined at least two preparations from a single stool, and as a rule it was only in difficult and doubtful cases that we examined more than two. It is highly probable, therefore, that a not inconsiderable number of examinations were made and recorded negative on stools that were really positive. In many cases this result seems to be due to the fact that the number of cysts in the faeces is really very small, but in other cases it is caused by the irregular distribution of cysts in the stools of infected patients. It is certain, then, that the percentages of infection we have recorded in Table IV are too low, and an attempt will be made later to ascertain approximately what was probably the real incidence of infection with the various protozoa, had all our cases been examined sufficiently often.

COMPARISON WITH THE RESULTS OF OTHER WORKERS

Since our findings have been obtained from the examination of a fairly large number of cases, it may be of some interest to compare them with the results of other workers. This can best be done by referring to Dobell's (1917) report to the Medical Research Committee on the protozoological investigation of dysentery cases and carriers. We wish at the outset to record our appreciation of the great value of his report, and to acknowledge the help we have received from its perusal. In Table VIII, p. 55, of that report are collected the results of the examinations made upon 7,021 cases from fourteen different centres. We will follow Dobell in confining our attention to those details which seem sufficiently reliable as a basis for certain deductions to be made. They are the results obtained from the cases examined at five centres, viz., Bristol, Chichester, Hornchurch, London Hospital, and Walton-on-Thames. The total number of cases examined amounted to 2,095 as compared with our

1,713, and the percentage of infection with *E. histolytica*, *E. coli* and *G. intestinalis* was 12·6, 40·5 and 16·1 respectively. From the analysis of our positive cases (Table IV), it will be observed that the percentages of infection found by us were 10·9, 29·4 and 18·5 for *E. histolytica*, *E. coli* and *G. intestinalis* respectively. Between Dobell's results and our own there is no very significant difference in the case of *E. histolytica* and *G. intestinalis*, but there is a rather decided difference in the percentage of cases infected with *E. coli*. We cannot suggest any explanation of this, for we have reason to believe that our methods of examination have been the same as those employed at the centres mentioned above, and our standard of non-infection has been practically the same.

DETAILED CONSIDERATION OF THE EXAMINATIONS MADE IN THE CASE OF EACH INFECTION SEPARATELY

We shall now pass to a consideration of the detailed records of cases infected with particular protozoa. In some instances it becomes necessary to distinguish between cases that have had treatment with emetine and those that have received no treatment. This distinction applies particularly to cases having an infection with *E. histolytica*, but we shall also make use of the distinction in considering the examinations made upon *E. coli* infections. Although *G. intestinalis* occurred frequently with *E. histolytica*, there is no evidence to show that treatment of the amoebic infection had any effect upon the flagellate. Since, therefore, our discussion of examinations made upon cases infected with *G. intestinalis* need not be complicated by any reference to the effect of emetine, we will begin our general treatment of the whole subject with a consideration of those records which relate to this flagellate.

A. EXAMINATIONS IN *G. INTESTINALIS* INFECTIONS

It is in connection with infections with this parasite that Dobell begins his critical study of the value of negative examinations. On account of the interest of detailed comparison between our figures and Dobell's, we have followed closely the arrangement of his report throughout. In order to obtain a ratio which will indicate how many examinations are necessary to detect the infection in the average infected case, he points out the importance of considering

only those cases upon which a large number of examinations have been conducted. Dealing with 18 cases which satisfy this condition (the average number of examinations per case was 51·5), he finds that in the average infected case the parasite will be found once in 4·5 examinations. It is important, also, in order to obtain a perfectly fair and representative sample of the infected material, that the cases should have been examined many times for some reason unconnected with their *G. intestinalis* infection. We do not think any case should be included if it has been examined many times *because* it was infected with *G. intestinalis*. Furthermore, it seems essential to have a large series of cases which satisfy the conditions mentioned. A small series may not be a fair sample of the total number of cases infected. We have at our disposal the records of thirty-nine cases infected with *G. intestinalis*, each of which has received at least twenty examinations. These cases have been chosen mainly from the present series, but they include a few that do not fall within the periods embraced by our reports. The cases were each infected with *E. histolytica*, and because of this the large number of examinations was made. Upon these 39 cases 1,448 examinations were conducted, or an average of 37 examinations per case. Positive findings were recorded 558 times, and negative findings occurred 890 times. On an average, therefore, the infection was detected once in 2·6 examinations. There is a considerable discrepancy between this and Dobell's result. We do not know the composition of his eighteen cases, but his ratio suggests that probably the greater number of them were cases which were mainly negative, and we are inclined to think that a larger number of cases is needed. Our own series of thirty-nine cases is probably too small, and we do not know that another thirty-nine examined similarly would give the same result for the average case. There is another consideration. If in any small series of cases it occurred that a few cases received many more examinations than the average number per case, and if these happened to fall at either extreme in the series, i.e., were mainly positive or mainly negative, the ratio for the average case would be accordingly affected. We are satisfied that the ratio we have obtained has not been affected in this way. Although our ratio suggests that the average infected case will be detected by three examinations, the real meaning of the ratio, or of

any ratio for any other protozoal infection, cannot be better expressed than in Dobell's words when he compares the examiner who wishes to detect a protozoal infection with a dice-thrower who wishes to throw aces. We consider the illustration so fitting that we quote the passage: 'As a die has six sides, the chances against an ace being thrown at any single throw are five to one; and on an average the ace will be cast only once in six throws. The dice-thrower must be prepared to make six throws for every ace; but he cannot always get an ace by casting the die six times, and he will often get one with less than six throws.'

Since many of the cases in our whole series of 1,713 had only two or three examinations each, and only a small proportion of cases had more than three, it is clear that the percentage of infection with *G. intestinalis* we have recorded (18·5) must be too low. Even in the diminishing numbers of cases we examined (see above) we find that the second examination had provided only 265 of the 317 infections ultimately found. At the end of the third examination 294 of the infected cases had been discovered, which means that 7·2 per cent. of the infections were detected after the third examination. In order to arrive at a figure which will more correctly represent the real incidence of infection among our cases, we should need to examine the total number of cases a large number of times each. We have records which suggest that twenty-eight examinations per case would have been necessary to make sure that no infection with *G. intestinalis* would escape detection. Since this number of examinations is quite impossible in practice when dealing with a large number of cases, we may select those few cases that have received many examinations each and ascertain the incidence of infection in these. For this purpose we have 110 cases, obtained mainly from the present series, but in part from the non-dysenteric cases investigated by Smith and Matthews (1917). They were each infected with *E. histolytica*, and for this reason each case received many examinations. Prolonged observation was not carried out in order to detect *G. intestinalis*. We have thus, for our present purpose, an unselected series which is likely to contain not only those cases of *G. intestinalis* that were frequently positive, but also those cases that were frequently negative and often not detected till a large number of examinations had been made. Our 110 cases

were examined 3,924 times, which is an average of 35·6 examinations per case. The minimum number of examinations on any case was 12; the maximum was 129. From an analysis of these 110 cases we obtain the following results regarding the incidence of infection with *G. intestinalis*:

TABLE V

Number of cases examined	Total number of examinations made	Number of examinations per case	Number of cases infected with *G. intestinalis*	Percentage of cases infected with *G. intestinalis*
110	330	3	19	17·2
110	660	6	22	20
110	3924	average of 35·6	29	26·3

The table shows that, by increasing the number of examinations made upon each case, the percentage of infection is raised. There is no evidence from this table that *G. intestinalis* has been found more frequently with *E. histolytica* than might have been expected. The result from three examinations per case gives, in fact, a lower percentage infection (17·2) than that recorded for our whole series of cases (18·5). But by increasing the number of examinations to six per case the percentage becomes 20, and with an average of 35·6 examinations per case the percentage of infection is raised to 26·3. It will be seen from the table that ten infections were discovered after the third examination. The particulars relating to these are as follows:

Infection was discovered at the 4th examination in 2 cases.
Infection was discovered at the 6th examination in 1 case.
Infection was discovered at the 7th examination in 1 case.
Infection was discovered at the 10th examination in 1 case.
Infection was discovered at the 12th examination in 2 cases.
Infection was discovered at the 13th examination in 1 case.
Infection was discovered at the 27th examination in 1 case.
Infection was discovered at the 28th examination in 1 case.

Since three of these ten cases were detected after the twelfth examination, it is not unreasonable to suppose that a few of the 110 cases were really infected with *G. intestinalis* which escaped

detection, for the minimum number of examinations upon any case was twelve, and the number of examinations necessary to detect the infection in a few cases was higher than this.

From the foregoing considerations, then, it seems probable that these 110 cases constitute a fair sample of our whole series in respect of their *G. intestinalis* infections. If this be so, the real incidence of infection with this flagellate among all our cases was certainly not lower than 26·3 per cent., and was perhaps as high as 30 per cent. Our recorded percentage (18·5), therefore, probably accounts for 60 to 70 per cent. of the real number of infected cases. This conclusion is supported by the result obtained by another method (see page 50). Our result is rather different from that finally reached by Dobell, who estimated that by a system of three examinations per case no more than 33 to 50 per cent. of *G. intestinalis* infections would be discovered. It may be recalled that the ratio we obtained to indicate what was the chance of detecting a *G. intestinalis* infection is higher than Dobell's, and our general experience is that *G. intestinalis* is not quite so sporadic in its occurrence as *E. coli*.

B. EXAMINATIONS IN *E. COLI* INFECTIONS

We shall now deal with the examinations made upon cases having an infection with *E. coli*. In many instances the infection was associated with *E. histolytica*. In cases having this double infection, we must bear in mind that the activity of the non-pathogenic species may have been affected by the treatment given specifically for the pathogenic. The coincident disappearance from the stools of both *E. histolytica* and *E. coli* cysts during treatment has occurred sufficiently often to render advisable a separate consideration of the *E. coli* infections, according to whether they were 'treated' or not.

(*a*) *'Untreated' Cases*

For the purpose of ascertaining the ratio of positive findings to the number of examinations made in untreated *E. coli* infections, we have the records of eighteen cases that have each been examined many times. With one exception these cases were followed *because* of their *E. coli* infection, and they therefore constitute a selected series. Upon these eighteen cases 409 examinations have been conducted, which is an average of 22·7 per case. No case was

examined fewer than eleven times. Cysts were discovered on 133 occasions, or, on the average, once in 3·1 examinations. This ratio is not markedly different from that found by Dobell (one positive in 2·7 examinations) for 'untreated' cases, but in view of the selection of the cases from whose records it has been derived, it may be too high. Many records of known positive cases show that three consecutive examinations could have been made with negative results, and this whether the case had been examined daily or at weekly intervals.

(*b*) *'Treated' Cases*

We have in this series fifty-seven cases entirely unselected, because each was also infected with *E. histolytica*, and for this reason each was kept under prolonged observation. Upon these fifty-seven cases 2,030 examinations were conducted, 552 of which were recorded positive for *E. coli*. On the average, therefore, the infection was detected once in 3·6 examinations. This ratio is only slightly different from that found for 'untreated' *E. coli* cases, and the ratio alone suggests that alcresta ipecac. adminstered for the *E. histolytica* infection had practically no effect on the non-pathogenic entamoeba. According to Low (1916), emetine hydrochloride given hypodermically produces only a temporary disappearance of *E. coli*. Dobell finds that emetine given in this way has no effect. He points out, however, that emetine bismuth iodide very frequently produces a negative period during and after treatment, but the effect is rarely permanent. To ascertain if alcresta ipecac. has in any instances an inhibitory effect upon the appearance of *E. coli* in the stools of infected patients, we have examined the individual records of the above fifty-seven cases in detail. In twenty-seven cases there was a disappearance of the organisms during treatment (the ratio of positive findings to number of examinations was 1 : 26·3); in twenty-one of these the infection appeared again at varying intervals after treatment, while in six cases there was no re-appearance at all. We have one instance of the cysts of *E. coli* being re-discovered on the thirty-sixth day after treatment for *E. histolytica* had stopped. Since the six cases were not examined for so long a post-treatment period as quoted, it is quite probable that continued examination would have shown a

reappearance of the infection. The records of the remaining thirty cases show that the infection of *E. coli* was quite unaffected by the alcresta administered for *E. histolytica*, the ratio during treatment being 1 : 3·3.

We shall now consider the incidence of infection with *E. coli* among our cases. Since the great majority of our cases did not receive so many as five or six examinations each, it is certain that the recorded percentage (29·4) of infection with *E. coli* is too low. In order to obtain a figure which will more correctly represent the real incidence of infection with this entamoeba, we shall follow the method we employed for *G. intestinalis* in this connection. It may be again pointed out that the 110 cases we make use of were examined a large number of times because they were each infected with *E. histolytica*. They were not examined in order to discover *E. coli*, but since each case had on an average 35·6 examinations, it seems probable that not only the frequently positive but also the majority of frequently negative *E. coli* cases were discovered in this series of 110. Of this number, 70, or 63·6 per cent., were finally found to be infected with *E. coli*. Had each case received only six examinations, only 58 infections would have been detected, or 52·7 per cent. If only three examinations had been made upon each case, the number found to be infected with *E. coli* would have been 47, or 42·7 per cent. These facts are presented in Table VI.

TABLE VI

Number of cases examined	Total number of examinations made	Number of examinations per case	Number of cases infected with *E. coli*	Percentage of cases infected with *E. coli*
110	330	3	47	42·7
110	660	6	58	52·7
110	3924	average of 35·6	70	63·6

We are here dealing with a constant number of cases, and it is clear that the increase in the number of cases found infected is the direct result of the increasing number of examinations conducted

upon each case. The twenty-three infections discovered after the third examination were recorded in the following order:—

Infection was discovered at the 4th examination in 7 cases.
Infection was discovered at the 5th examination in 3 cases.
Infection was discovered at the 6th examination in 1 case.
Infection was discovered at the 7th examination in 4 cases.
Infection was discovered at the 8th examination in 1 case.
Infection was discovered at the 12th examination in 2 cases.
Infection was discovered at the 14th examination in 1 case.
Infection was discovered at the 24th examination in 1 case.
Infection was discovered at the 30th examination in 2 cases.
Infection was discovered at the 45th examination in 1 case.

It will be seen from these details that no fewer than five infections were discovered after the twelfth examination, and since the minimum number of examinations on any case was twelve, it is not impossible that among our 110 cases some were infected with *E. coli* which escaped detection. The actual percentage of infected cases found (63·6 per cent.) may therefore be a little too low, but even 63·6 per cent. is strikingly different from that recorded as the incidence of infection with *E. coli* (29·4 per cent.) among the total number of cases examined. We do not believe that the difference between 29·4 per cent. and 63·6 per cent. can be entirely accounted for by prolonged observation of the cases, for even at the third examination our 110 cases have a much higher percentage (42·7) of *E. coli* than our whole series of cases which received an average of three examinations each. The figures suggest, in fact, that *E. coli* infections tend to occur more frequently with *E. histolytica* than would be expected from a random association of the two protozoa.

If we suppose that the percentage figures given in Table VI for the *E. coli* infections among the 110 selected cases remain at each stage about 13 per cent. higher than they would be for a series of unselected cases, we obtain the following results as the probable real incidence of infection with *E. coli*:—With 3 examinations per case 30 per cent., with 6 examinations 40 per cent., and with an average of 35·6 examinations per case 50 per cent. We are able to support these figures by referring to a few cases infected with *G. intestinalis* that have received a fairly large number of examinations. Since there is no evidence that *E. coli* is particularly associated with *G. intestinalis*, the cases for our present purpose may

be regarded as quite unselected. They were not infected with *E. histolytica*. There are 45 cases in all examined at least 7 times each and on an average 11·6 times. Ultimately 23 cases, or 51 per cent., were found to be infected with *E. coli*. Had each case been examined only 6 times the percentage would have been reduced to 42·2, and with only three examinations made upon each case, the percentage of *E. coli* would have been 31·1. We ought to note that these results have been obtained with an average of 11·6 examinations per case as compared with 35·6 for the 110 *E. histolytica* cases.

From the foregoing considerations, then, we conclude that the real incidence of infection with *E. coli* among all our cases was 50 per cent. to 55 per cent. It follows, therefore, that our recorded percentage of 29·4 accounts for 55 per cent. to 60 per cent. of the real number infected. These figures agree remarkably closely with those given by Dobell, who comes to the conclusion that in his series of cases no more than 50 per cent to 66 per cent. of the infections with *E. coli* were detected.

C. EXAMINATIONS IN *E. HISTOLYTICA* INFECTIONS

(*a*) *Untreated cases*

In our series of 1,713 cases we have none infected with *E. histolytica* that have remained, so far as we know, without treatment, but we may refer to certain cases found by Smith and Matthews (1917) in their investigation on non-dysenteric patients. There are at our disposal 10 cases whose records are sufficiently long to be of value. Each case was examined at least 10 times, and the total number of examinations made amounted to 236. Of these 105 were recorded positive and 131 were recorded negative. The ratio of positive findings to the number of examinations made is thus 1 : 2·25. It is almost certain that this ratio is too high, for it is derived from cases that were selected because of the early detection of the infection. This is proved by the fact that 9 of the 10 cases were found positive at the first examination, and in the remaining case the infection was discovered at the third examination. We are thus dealing with cases that were likely to remain more positive than negative however long examined. There are only two outstanding exceptions: in one a positive examination was followed by 26 negative, and in the other a positive examination was

followed by 31 negative. Such instances as these show incidentally that three or more consecutive negative examinations can be made on the stools of a patient really infected. This possibility exists indeed in cases whose records are even more positive than negative.

We do not know how often three 'negative' examinations are recorded for cases that are really positive, but such occurrences are doubtless not infrequent, and consequently introduce an appreciable error into any records based on three examinations only. Such an error must in fact have occurred not infrequently in the full series of results set out in the table below, and the table itself shows that an appreciable number of *E. histolytica* infections were not detected until after the third examination, even when diminishing numbers of cases were examined. Table VII shows the increase and actual number of *E. histolytica* cases discovered at each examination.

TABLE VII

Examination	No. of cases examined	Total No. of cases infected with *E. histolytica*	Percentage of total cases examined	Actual No. of new *E. histolytica* cases found	Percentage of total *E. histolytica* cases found
First	1713	101	5·9	101	53·7
Second	1560	144	8·4	43	22·9
Third	1093	173	10·1	29	15·4
Fourth	459	183	10·6	10	5·3
Fifth	301	185	10·8	2	1·1
Sixth	236	188	10·9	3	1·6
				188	100·0

It will be seen from the table that 15 cases infected with *E. histolytica* were discovered after the third examination. This number is 8 per cent. of the total number of *E. histolytica* cases found, and raises the incidence of infection with this parasite from 10·1 to 10·9. It seems certain that if all our cases had been examined at least six times each, the percentage would have been considerably higher than that recorded. A fuller study of the figures in the table leads us to believe (see page 54) that probably 20 per cent. to 23 per cent. were really infected. Our figures

suggest, then, that a system of three examinations per case, will lead to the discovery of no more than about 50 per cent. to 55 per cent. of all the cases that are harbouring *E. histolytica*. This figure is very similar to that found for *E. coli* infections.

(*b*) *Treated cases*

Since the examinations made upon treated cases are considered in detail in another part of this report, they will be referred to here only briefly. While in untreated cases positive and negative findings were recorded at random, they were not so recorded in cases undergoing treatment. As a rule these cases responded to emetine (in the form of alcresta ipecac. or biniodide), at any rate to the extent of becoming negative a few days after treatment began. This negative period was in many cases prolonged after treatment stopped, and cases were considered fit for discharge when the examinations continued negative during a post-treatment period of two to three weeks. In a certain number of cases the negative period during treatment has been broken by sporadic 'positive' days, and there have been one or two cases which did not become consecutively negative until treatment was over, or almost over. As a rule, however, the period of treatment was negative, even in cases which afterwards relapsed. It follows, therefore, that both in cases that were cured as well as in the majority of those that relapsed the number of negative to positive findings was high. But in a few instances the infections behaved more like certain untreated cases, where positive examinations were recorded as frequently as negative. Such resistant cases remained under observation for long periods. One case was found positive 63 times and negative 59 times; another had 90 positive examinations and 29 negative.

D. EXAMINATIONS IN *C. MESNILI* INFECTIONS

The number of cases we found infected with this flagellate was small, and this is no doubt partly the result of our examinations having been conducted mainly upon formed or semi-formed stools. Wenyon and O'Connor (1917) have pointed out that the free forms and even the cysts of this flagellate are found more abundantly (in infected cases) in unformed stools. 'With Trichomonas, and to a lesser extent with Tetramitus, which has a recognizable encysted stage, there is difficulty of recognition in the formed stool. . . Flagellate infections

were only recognised in many cases after the patients had been treated with salines.' We have only 19 cases that have been under continued observation. The majority of the cases were mainly negative cases, and it is not surprising, therefore, when we look at individual records, to find that the first three examinations of our cases provided a very low percentage of infection with this flagellate. To return to the 110 *E. histolytica* cases that have been examined on an average 35·6 times each, we obtain the following results regarding their number infected with *C. mesnili*.

TABLE VIII

Number of cases examined	Total number of examinations made	Number of examinations per case	Number of cases infected with *C. mesnili*	Percentage of cases infected with *C. mesnili*
110	330	3	2	1·8
110	660	6	6	5·4
110	3924	average of 35·6	15	13·6

We have recorded 3·5 per cent. as the incidence of infection for our whole series of 1,713 cases. The percentages given in the above table, if only three or six examinations per case had been made, do not seem inconsistent with that figure. The surprising increase from 5·4 per cent. to 13·6 per cent. is the result of the prolonged observation of the cases which incidentally gave a greater chance of making examinations on those occasional days when more liquid stools were passed. The finding of infections of *C. mesnili* after the third examination is even more remarkable than in the case of *E. coli* infections. The details of the thirteen cases recorded in Table VIII, whose infections were not discovered till after the third examination, are as follows:—

Infection was discovered at the 4th examination in 2 cases.
Infection was discovered at the 5th examination in 2 cases.
Infection was discovered at the 7th examination in 1 case.
Infection was discovered at the 9th examination in 1 case.
Infection was discovered at the 12th examination in 1 case.
Infection was discovered at the 16th examination in 1 case.
Infection was discovered at the 17th examination in 1 case.
Infection was discovered at the 21st examination in 2 cases.
Infection was discovered at the 23rd examination in 1 case.
Infection was discovered at the 44th examination in 1 case.

From these details it will be seen that a very considerable proportion of the infections were not detected until a large number of examinations had been performed. As many as six were discovered after the twelfth examination. It is not impossible that *E. histolytica* cases do not form a fair sample of our whole series with regard to infection with *C. mesnili*, but the main fact to be deduced from the figures we have given is the large number of examinations necessary to discover even half the number of infected cases, and it is not improbable that the real incidence of infection among our total series of cases was 12 to 15 per cent.

E. EXAMINATIONS IN *T. INTESTINALIS* INFECTIONS

We have found this flagellate in only 1 per cent. of all the cases we have examined. But it is certain that this protozoon is found most frequently in diarrhoeic conditions of the stool, and this fact helps to account for the small number of cases in which we found the organism. Our low percentage may also be partly due to the insufficient number of examinations conducted upon many of our cases. Thus, out of six infections found among 110 *E. histolytica* cases three were detected after three examinations per case had been made. Of the other three, one was discovered at the fourth examination, one at the eleventh and one at the thirty-second. In these 110 cases, then, the percentage of infections was ultimately 5·4 instead of 2·7, as it would have been if three examinations had been taken as the standard of non-infection.

We have been struck by the infrequency of the occurrence of *T. intestinalis* compared with other flagellates in the stools of patients known to be infected. Six cases examined 234 times were found positive on only 23 occasions. Three of these cases were each positive once out of 15, 25 and 79 examinations respectively. Whether the cases had lost their infection or whether the condition of the stools was connected with the rare appearance of the flagellate, we are unable to say.* Upon one case—not recorded in our series of hospital cases—49 examinations were made, 39 of which were recorded positive. In this instance, we know that the stools

* We may mention that since the above was written *Trichomonas* has been rediscovered in the third case referred to. The reappearance of the flagellate occurred at the 116th examination, and the interval between the two findings extended to about 28 weeks. In this particular case, then, the infection had apparently not been lost during this long period.

were regularly of a diarrhoeic nature. We may mention that this case was examined regularly (almost daily) to ascertain if any periodicity could be discovered in the occurrence of the flagellate. The infection was generally heavy, and no attempt was made to estimate accurately the number of flagellates present each day, but by a rough method of counting we found that on different days they occurred in numbers ranging from 2 to 120 per sq. mm. of preparation. During the period of our observations there occurred 10 days on which no flagellates were found. These 'negative' days occurred at quite irregular intervals, and when all the figures are plotted there is no evidence of any periodicity in the occurrence of this flagellate in the stools. We have not sufficient data to enable us to discuss the question of periodicity for other protozoa, in which, as a rule, both the free forms and the cysts have to be taken into account.

Our method of denoting the relative abundance of an infection has been a use of the plus sign. Thus + meant a scanty infection, ++ indicated a fairly heavy infection, and so on. This procedure, though of considerable value, was obviously not suited for the solution of a problem which demands exactness in the methods employed. Further, our records were defective, because we have none in which examinations were recorded daily without intermission. Although, therefore, our best records do not suggest a definite periodicity for any of the protozoa with which we have dealt, we do not claim to have made a critical investigation of the matter.

PART III.—STATISTICAL CONSIDERATION OF THE COMBINED RESULTS

In our First Report, p. 423, we gave curves showing the increase in the number of infections found by continued examination of the cases dealt with in that report. We there stated that the curves were merely another way of presenting the actual results of our examinations. They were not to be taken as giving any guidance as to the number of examinations necessary to detect the great majority of the various infections. The curves and the figures they represented suffered from the defect that they were the result of the examination of constantly decreasing numbers of cases. Especially

at the fifth and sixth examinations, the numbers examined were too small to give any true idea of what would be the result of continuing the examination of the full number of cases up to those later stages. We have now at our disposal the results of the examination of much larger numbers of cases, and we propose in this part of the paper to use these figures to give us an idea of (1) the true incidence of the three main infections, *G. intestinalis*, *E. coli* and *E. histolytica*, in our series of cases, and (2) the relative value of the examinations from the first to the sixth in detecting the various infections. In order to do this, we will complete our investigation—incompletely sketched in the First Report, pp. 424 and 425—into the course of the ideal curves which would represent the findings if *every* case could receive a definite number of examinations. The number of cases dealt with in the present report is 1,713, and we shall therefore try to obtain the numbers of infections which should be found if every one of these 1,713 cases could have received six examinations.

INFECTIONS WITH *G. INTESTINALIS*

We will begin by considering the infections with *G. intestinalis*. Table IX (column 1) gives the numbers of infections of this flagellate actually found at each examination from the first to the sixth when diminishing numbers were examined. After each examination a considerable number of cases was dropped and did not reappear for further examination. We have data showing the composition of these dropped cases, i.e., how many were (*a*) positive and (*b*) negative for *G. intestinalis* up to that stage. We know, therefore, how many positives and how many negatives came up for further examination, and we also know how many new infections with *G. intestinalis* were found at each stage. These new infections were found, of course, among the negative cases examined, and it is easy to calculate by simple proportion what numbers of new infections should have been found among the negatives which were not re-examined. The only assumption made in the calculation is that the negatives not examined are similar in composition to those examined, i.e. that the material is homogeneous, and this assumption is, we believe, amply justified. Table IX (column 2) gives the result of such a calculation for the infections of *G. intestinalis*.

It appears from this calculation that 23 per cent. of all the cases examined would have been found to be infected with *G. intestinalis* if every case could have been examined six times. We cannot by this method calculate what would be the final percentage of cases infected with *G. intestinalis* if each case were examined many times. Since, however, among the comparatively small number of cases carried further than the sixth examination there were found nine further infections of *G. intestinalis*, one occurring so late as the

TABLE IX

Examination	Numbers actually examined	Total *G. intestinalis* cases actually found from diminishing numbers examined	Total *G. intestinalis* cases if all cases had six examinations (calculated)	Col. (2) as percentage of all cases examined (1713) (with probable error)*	Col. (2) as percentage of cases to be found at end of six examinations
		(1)	(2)	(3)	(4)
First	1713	194	194	11·3 ± ·61	48·7
Second	1560	265	272	15·9 ± ·71	68·3
Third	1093	294	318	18·6 ± ·75	79·9
Fourth	459	300	342	20·0 ± ·77	85·7
Fifth	301	304	367	21·4 ± 80	92·2
Sixth	236	308	398	23·2 ± ·82	100·0
Finally	—	317	—	—	—

twenty-eighth examination, it seems reasonable to estimate that *G. intestinalis* actually occurred in about 30 per cent. of all the cases. This estimate (30 per cent.) is in agreement with the figure obtained on p. 39, where reasons are given for thinking that the real incidence of *G. intestinalis* infections is rather higher than 26 per cent.

As regards the reliability of the figures in Table IX, it may be

* The term *probable error* is here used in its usual significance in statistical work. For example, the percentage of *G. intestinalis* found in the first examination, as shown in Table IX, being 11·3 ± ·61, any further observed value of this percentage is as likely to fall within the limits 10·69 and 11·91 as outside those limits. When the limits are narrow, as in the present case, and when any further observation is just as likely to fall within them as in the very wide range of possible values outside them, a reliable series of observations is indicated.

stated that even at the sixth examination as many as 175 cases, so far negative for *G. intestinalis*, were re-examined, and thus the smallest number on which our calculations are based was sufficient to provide a reliable sample of the whole series of cases. Mr. W. Stott, Honorary Statistician to the Liverpool School of Tropical Medicine, to whom we tender our sincere thanks, has kindly calculated for us the probable error of the percentages given, and it will be seen from Table IX (column 3) that the figures are satisfactory.

Table IX (column 4) also provides a ready means of estimating the relative value of each of the earlier examinations in terms of the sixth. Six examinations is the highest number which has so far been advocated (by Dobell, 1917), and it will probably be considered the maximum which is practically attainable for all cases. It will be seen that anyone who is content to miss 14 per cent. of the infections of *G. intestinalis* to be found by six examinations need only conduct four examinations, if willing to miss 20 per cent. of such infections, he may fix on three examinations per case as a sufficient number, and so on. Such considerations as these will have more importance when we come to consider the infections of *E. histolytica*.

Summarising this section, we may say that the real incidence of *G. intestinalis* infections in our cases is about 30 per cent., and that about 23 per cent. would be found by a system of six examinations. It follows from our figures that three examinations should discover about 60 per cent. of all the cases harbouring *G. intestinalis*, and about 80 per cent. of those to be found by six examinations per case. Comparing these figures with those of Dobell (1917), p. 57, we find that he estimates the real incidence of infections of *G. intestinalis* in his series of cases to be 30 to 45 per cent., and that only 33·3 to 50 per cent. of these are found by a system of three examinations. We have previously (p. 39) commented on the discrepancy between our figures for *G. intestinalis* and his. The table just given only brings out the difference in another form.

INFECTIONS WITH *E. COLI*

We will next consider the infections with *E. coli*. Table X, which is obtained in an exactly similar way to Table IX, sets forth the results of our findings for *E. coli*.

TABLE X

Examination	Numbers actually examined	Total *E. coli* cases actually found from diminishing numbers examined	Total *E. coli* cases if all cases had six examinations (calculated)	Col. (2) as percentage of all cases examined (1713) (with probable error)	Col. (2) as percentage of cases to be found at end of six examinations
		(1)	(2)	(3)	(4)
First	1713	265	265	15·5 ± ·70	40·5
Second	1560	392	406	23·7 ± ·82	62·1
Third	1093	457	510	29·8 ± ·89	78·0
Fourth	459	478	595	34·7 ± ·92	91·0
Fifth	301	484	635	37·1 ± ·94	97·1
Sixth	236	486	654	38·2 ± ·94	100·0
Finally	—	505	—	—	—

It will be seen that the *E. coli* cases which should be found by six examinations form 38 per cent. of the total cases examined. As in the case of *G. intestinalis*, this method only allows us to make a rough estimate of the real incidence of *E. coli* cases, i.e. the number of infections of *E. coli* which would finally be found if each case received very many examinations. Considering that 19 infections of *E. coli* were found in the comparatively small number of cases examined more than six times, it seems reasonable to suppose that the final figure should be about 50 per cent., especially since this figure agrees with that obtained by a different method in Part II, p. 43.

Even at the sixth examination 123 cases previously negative for *E. coli* were examined, a number sufficient to give a reliable basis for calculation. The probable error of the percentages is given in Table X (col. 3), and shows that the figures may be considered satisfactory.

Table X (col. 4) gives for *E. coli* infections the relative value of each examination in terms of the sixth. It will be seen that though the first and second examinations are relatively less important than are the same examinations in the case of *G. intestinalis*, yet by the

end of the third examination there will have been found about 80 per cent. (78 per cent. exactly) of the infections to be expected from six examinations. This is the same figure as we obtained at this stage for infections with *G. intestinalis*.

Summarising this section, we may say that the real incidence of *E. coli* infections in our cases is about 50 per cent., and that about 38 per cent. would be found by a system of six examinations. It follows that three examinations should discover about 60 per cent. of all the cases infected with *E. coli*, and about 80 per cent. of those to be found by six examinations. Both these percentages are the same as the corresponding figures found by us for *G. intestinalis* infections. The figures given by Dobell (1917) (Report, p. 57) are, for real incidence of *E. coli* infections 60 to 80 per cent., and for the proportion of these to be found by a system of three examinations per case 50 to 66·6 per cent. Our figure (60 per cent.) for the proportion to be found by 3 examinations agrees well with Dobell's, lying about midway between his two extremes. The percentage we have reached as expressing the real incidence of *E. coli* infections (50 per cent.) differs somewhat widely from his, being about 20 per cent. lower. We may note that our findings are throughout lower than his. At the first examination, at the third, at the sixth, and right through the series, our figures remain from 10 to 20 per cent. lower. This is a fact which we cannot explain. We have already commented on it on p. 35

INFECTIONS WITH *E. HISTOLYTICA*

We consider the infections with *E. histolytica* last, partly because they are important from the practical point of view, and in this position they lead directly on to the practical discussion which follows, and partly because, as our figures are not quite so reliable as those for *G. intestinalis* and *E. coli*, we need to check our statements about *E. histolytica* infections by reference to the results for the two protozoa previously considered. The reliability of the figures is somewhat impaired by the fact that, as a rule, only those cases infected with *E. histolytica* received as many as six or more examinations, and thus there was a tendency for cases negative for *E. histolytica* to be dropped out before the later examinations were reached. In spite of this, however, we had before us at the sixth

examination 101 cases so far negative for *E. histolytica*, and, though larger numbers are desirable, yet this number ought to provide a reasonably good sample of all the material. Actually out of the 101 cases three new infections of *E. histolytica* were found. The figures given for the probable error in Table XI (column 3) show that considerable confidence can be placed in the general results.

Table XI gives similar figures for *E. histolytica* to those which have already been given for *E. coli* and *G. intestinalis*.

TABLE XI

Examination	Numbers actually examined	Total *E. histolytica* cases actually found from diminishing numbers examined	Total *E. histolytica* cases if all cases had six examinations (calculated)	Col. (2) as percentage of all cases examined (with probable error)	Col. (2) as percentage of cases to be found at end of six examinations
		(1)	(2)	(3)	(4)
First	1713	101	101	5·9 ± ·45	33·4
Second	1560	144	148	8·6 ± ·54	49·0
Third	1093	173	195	11·4 ± ·62	64·6
Fourth	459	183	245	14·3 ± ·68	81·1
Fifth	301	185	273	15·9 ± ·71	90·4
Sixth	236	188	302	17·6 ± ·74	100·0
Finally	—	188	—	—	—

It will be seen that the *E. histolytica* cases which should be found by six examinations form about 18 per cent. of the total cases examined. We find it particularly difficult to estimate from these figures the final incidence of *E. histolytica* infections among the cases we examined. We did not find any case of *E. histolytica* after the sixth examination, although 90 cases were examined at least a seventh time. Our not finding any new infection of *E. histolytica* at the seventh examination was, however, probably a mere chance, and we see no reason to doubt that if a good number of non-*histolytica* cases could be examined many times we should obtain

from them *E. histolytica* cases at examinations much later than the sixth, as we actually did for infections both of *E. coli* and *G. intestinalis*.

In spite of the difficulty presented by the fact that we obtained no new infections of *E. histolytica* after the sixth examination, we may be able to fix with some approach to accuracy the limits between which the real incidence of *E. histolytica* infections must lie. Since Table XI points to about 18 per cent. as being the figure which should be reached already at the sixth examination, we can scarcely suppose that the final incidence after a large number of examinations could be less than 20 per cent., and we may take this as the lower limit. For the upper limit we may use the figures we have reached for infections of *E. coli*. It is shown on p. 52 that the incidence of infections of *E. coli* at the sixth examination is 38 per cent., and we give reasons for thinking that it probably amounts finally to 50 per cent. If the figure of 17·6 per cent. for *E. histolytica* at the sixth examination is increased in the same ratio, we get 23 per cent. as the final incidence of *E. histolytica* infections. Now the evidence seems to point to the probability that *E. histolytica* is not *more* likely to occur in these later examinations than *E. coli*. If it were, we could hardly have got no new infections of *E. histolytica* on examining 90 cases when we found six new infections of *E. coli* at the seventh examination, the numbers examined being about the same. We may, therefore, take 23 per cent. as the upper limit of the real incidence of infection with *E. histolytica*, and thus obtain the figures 20 to 23 per cent. as expressing this incidence with a fair approach to accuracy. These figures agree closely with those given by Dobell, 18 to 25 per cent.

Table XI (column 4) gives for *E. histolytica* infections the relative value of each examination in terms of the sixth. It will be seen from the figures that the earlier examinations provide a smaller and therefore the later examinations a larger proportion of the cases to be expected by six examinations than was the case in *E. coli* and *G. intestinalis* infections. The third examination, for instance, provides only 65 per cent. of the infections to be found by six examinations.

Summarising for *E. histolytica* infections, then, we may say that the real incidence of these among the cases examined by us

was from 20 to 23 per cent., and that about 18 per cent. would have been found by a system of six examinations. It follows that three examinations should discover 50 to 57 per cent. of all the cases infected with *E. histolytica*, and 65 per cent. of those to be found by six examinations. The estimates for the final incidence of *E. histolytica* infections and for the proportion of these to be found by three examinations are in fairly close agreement with those given by Dobell (1917).

NUMBER OF EXAMINATIONS RECOMMENDED IN PRACTICE

We now come to consider the important practical problem as to how many examinations for intestinal protozoa should be carried out on all cases of dysentery. In our First Report we contented ourselves by stating that fewer than three examinations could upon no consideration be thought sufficient. We did not express any opinion as to whether three examinations were sufficient, since we had not then a broad enough basis of facts from which to form an opinion as to comparative value of the later examinations. With the fuller figures before us, we conclude that a system based upon three examinations per case is clearly inadequate. It appears at the most to detect about 60 per cent. of all the cases infected with *E. histolytica*, and considering the importance of the detection of infections of this dangerous parasite, it is inadmissible that so low a standard should be permanently adopted. Whether for three examinations, four, five, or six should be substituted is a matter for the serious consideration of the medical authorities of the army. Six examinations is, as we have said, the highest number that has been advocated, and probably the highest that is at all likely to be carried out in practice. There is no doubt that from the point of view of detecting a considerable proportion of all the *E. histolytica* cases, six is extremely desirable. If for any reason concerned with practical difficulties a smaller number of examinations should be decided on, then our Table XI gives a fairly accurate idea of the risks taken by omitting the sixth, or the fifth and sixth examinations, or in remaining content as at present with three examinations only. It appears that five examinations would be likely to give us about 90 per cent., four examinations about

80 per cent., and three examinations 65 per cent. of the cases to be found by six examinations.

On another important practical problem, viz., the period over which the examinations should be extended, we can at present say very little. We may be able later to offer evidence upon it. We will only say here that we know of no evidence which would point to six daily examinations being less efficient than six weekly examinations. On the other hand, we are not prepared to say that they are equally efficient. Obviously the settling of this question is a matter of great practical importance.

We have obtained some concrete evidence as to the accuracy of the calculations set forth in the Tables IX, X and XI. By the great kindness of Mr. W. O. Redman King, who went to considerable trouble for us, we have obtained records of certain findings made at Barton-on-Sea. These chiefly refer to cases examined by us twice and receiving their third examination at Barton-on-Sea. There were 250 such cases. From our table it will be seen that there were 620 cases dropped by us at the first and second examinations which should have received a third, and we calculate that they would have provided us with 24 new *G. intestinalis* infections, 53 new *E. coli* infections, and 22 new *E. histolytica* infections. At this rate, the 250 examined by Mr. Redman King should have provided 10 new infections of *G. intestinalis*, 21 of *E. coli*, and 9 of *E. histolytica*. Mr. King actually found among them 7 new *G. intestinalis*, 22 new *E. coli*, and 10 new *E. histolytica* infections. These findings confirm the accuracy of our calculations, at any rate for that particular examination.

We give in Fig. 1 a graphical presentation of the results just outlined for the three main infections. The three curves represent the figures in column 3 of our Tables IX, X and XI.

We have indicated in Fig. 1 by a horizontal line our estimate of the real incidence of each infection, viz., 50 per cent. for *E. coli*, 30 per cent. for *G. intestinalis* and 23 per cent. for *E. histolytica*. In each case the curve of findings gradually and at a constantly diminishing rate approaches this limiting line. The two would practically come into contact at some remote examination, e.g. the fiftieth. It seems to us that the general form of these curves and

was from 20 to 23 per cent., and that about 18 per cent. would have been found by a system of six examinations. It follows that three examinations should discover 50 to 57 per cent. of all the cases infected with *E. histolytica*, and 65 per cent. of those to be found by six examinations. The estimates for the final incidence of *E. histolytica* infections and for the proportion of these to be found by three examinations are in fairly close agreement with those given by Dobell (1917).

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80 per cent., and three examinations 65 per cent. of the cases to be found by six examinations.

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their relationship to the limiting lines confirm our belief in the correctness of the estimates we have made of the real incidence of each infection.

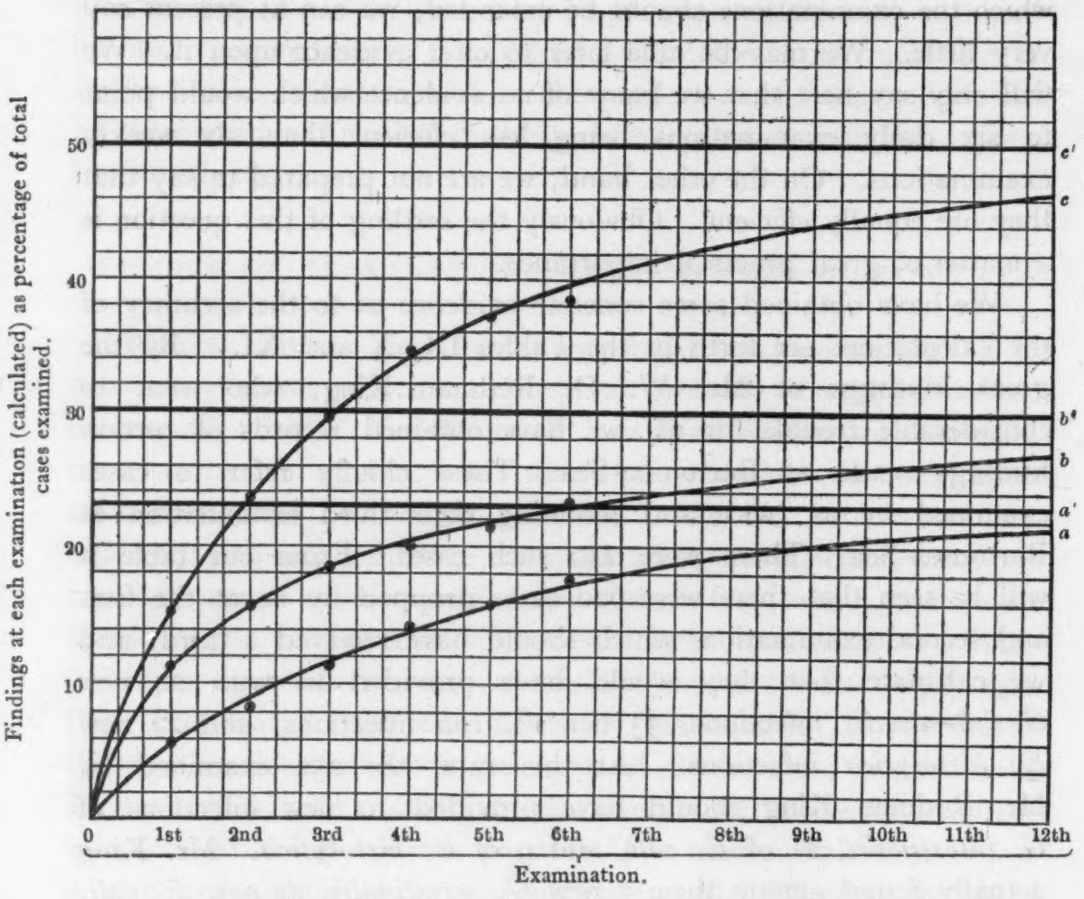


FIG. 1.—Curves showing the calculated percentage increase in infections at each of six examinations for (*a*) cases infected with *E. histolytica*; (*b*) cases infected with *G. intestinalis*; and (*c*) cases infected with *E. coli*. Estimates of the real incidence of infection with (*a'*) *E. histolytica*; (*b'*) *G. intestinalis*; and (*c'*) *E. coli*.

CONCLUSIONS DERIVED FROM PARTS II AND III

1. In a series of 39 cases infected with *G. intestinalis* (mean number of examinations per case 37) the parasite was found on an average once in three examinations (exactly 1 : 2·6).

2. A system of three examinations per case should result in finding that 18 to 19 per cent. of all the cases examined are infected with *G. intestinalis*. Six examinations per case should give 23 per cent., and ultimately 30 per cent. should be found. Thus

three examinations per case should discover 60 per cent. of the total number of *G. intestinalis* infections, and 80 per cent. of those to be found by six examinations per case.

3. Among 57 cases infected with *E. histolytica* and *E. coli* the latter was found on an average only once in 3·6 examinations. In 30 of these cases alcresta ipecac. given for *E. histolytica* had no effect upon *E. coli*, which was found among these 30 cases during the periods of treatment on an average once in 3·3 examinations. In the other 27 cases alcresta had probably an inhibitory effect upon *E. coli*, for it was discovered during the periods of treatment on an average only once in 26·2 examinations.

4. A system of three examinations per case should result in finding that 30 per cent. of all the cases examined are infected with *E. coli*. Six examinations per case should give 38 per cent., and ultimately 50 to 55 per cent. should be found. Thus three examinations per case should discover about 60 per cent. of the total number of *E. coli* infections and 78 per cent. of those to be found by six examinations per case.

5. Three 'negative' examinations may frequently be recorded upon cases really infected with *E. histolytica*. One case was found positive only once in 32 examinations.

6. A system of three examinations per case should result in finding that 11 to 12 per cent. of all the cases examined are infected with *E. histolytica*. Six examinations per case should give 18 per cent., and ultimately 20 to 23 per cent. should be found. Thus three examinations per case should discover 50 to 57 per cent. of the total number of *E. histolytica* infections and 65 per cent. of those to be found by six examinations per case.

7. It is recognised that for the purpose of detecting the great majority of infections of *E. histolytica* a system of six examinations per case is extremely desirable.

PART IV.—TWENTY CASES OF INFECTION WITH *ENTAMOEBA HISTOLYTICA* FROM THE FRENCH FRONT

To the worker engaged in the detection and cure of carriers of *Entamoeba histolytica*, it is often pointed out that, in a well-ordered and well-drained community the risk to the civilian population of infection with tropical dysentery brought from the Mediterranean

area and Mesopotamia by soldiers is so small as to be negligible. There is a good deal, no doubt, to be said for this view, although it seems to us still too early to be confident that, with the disbanding of large numbers of troops, the risk will always remain so small.

But even the most energetic objector to the spending of time and trouble in the detection of carriers, is bound to admit that the sending of such men to live among their fellows in the insanitary conditions imposed by trench warfare is a very different matter, and fraught with real and obvious danger to healthy troops.

It will be agreed that amoebic dysentery is a disease not endemic in Northern France; yet there have been a number of cases of dysentery in the armies there during the past year, in which the causal organism was apparently *Entamoeba histolytica*. The question has aroused a good deal of interest, and the French medical journals, in particular, have published detailed accounts of cases of amoebic dysentery and liver-abscess occurring in French soldiers on the Western front, who had never been out of France before the war. It seems reasonable to conclude that such cases were infected in France by carriers, who had acquired their infection in tropical or sub-tropical regions, either during the present war, or during travel earlier in their lives.

There have come under our notice during the last six months, and mainly during the last three, among the cases invalided from France,[1] twenty 'carriers' of *E. histolytica*, most of them with a history of dysentery. We give below the brief 'histories' of these twenty men, our object being to discover whether they had actually suffered from dysentery in France, and whether they had ever been out of England before going to France.

Case 1*[2]. This man was invalided home from France with shell-shock in August, 1916. On examination his stools were found to contain *E. histolytica* cysts. He said that he had never suffered from diarrhoea or dysentery in his life. Before he joined the army, he had been a miner in Yorkshire, and had never been out of England.

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1. It is only in the Tropical School Auxiliary Military Hospital and the Royal Infirmary that we can make a *regular* practice of getting the histories of cases examined by us. It is probable, therefore, that other cases from France were among the positive cases recorded from other hospitals in the Western Command.

2. Cases marked with an asterisk are 'non-dysenteric' cases, two of which (Cases 1 and 2) have been described already by Malins Smith and Matthews (1917), and the other four (Cases 3, 8, 9, and 18) will appear in a later report on non-dysenteric cases.

Case 2*. Was invalided home from France with wounds, in October, 1916. He had spent thirteen months with the army in France, and had never been out of England before, where he was a miner in the north. He had no record of dysentery, and stated that he had never in his life suffered from diarrhoea or dysentery. Nevertheless, *E. histolytica* cysts were found in his stools.

Case 3*. This case was invalided from France. *E. histolytica* cysts were found in his stools. He had no history of dysentery, but said that he had suffered from diarrhoea for the first time in his life in France in July, 1916. He had spent eight months in France, and had never been out of England before. He was treated with alcresta ipecac., and was cleared of cysts before he was discharged on the 4th January, 1917.

Case 4. This case went sick with dysentery at the Somme, France, on 8th September, 1916. He was invalided home, and *E. histolytica* cysts were detected in his faeces. He had never been out of England before going to France with the army. After treatment with alcresta and biniodide, he was discharged as cured in the third week of January, 1917.

Case 5. Developed dysentery at the Somme, France, in September, 1916. He was sent to Liverpool where *E. histolytica* cysts were found in his stools. He was treated with alcresta, and when discharged on the 14th January, 1917, was no longer passing cysts. He stated that he had never been out of England before going to France.

Case 6. Sent home with dysentery, contracted at Etaples, France, 1st October, 1916. He was never in the front line trenches. *E. histolytica* cysts were found in his stools. He was treated with alcresta and was discharged as cured on the 10th January, 1917.

Case 7. This man also contracted dysentery on the Somme, in September, 1916. Cysts of *E. histolytica* were detected by us in his stools. He was treated with alcresta, and was discharged as cured on the 3rd January, 1917. He had never been out of England before he went to France.

Case 8*. Invalided with wounds from France. Cysts of *E. histolytica* were found in his stools. Questioned, he said that he had been very bad with diarrhoea in France in December, 1915; he was ill then for a fortnight, with 10-15 stools a day. He had never been out of England before, except for one fortnight before the war, spent in Bruges and Ostend.

Case 9*. This man went to France on the 18th July, 1916, and returned to England with pneumonia on the 12th August. He was examined by us for intestinal protozoa, and cysts of *E. histolytica* were found. He had no history of dysentery, and declares that he never had any diarrhoea. He has never been out of England before, and was no further south than the Somme.

Case 10. This case was invalided from Salonica, in November, 1916. But he stated that this was only a relapse, and that he had had dysentery in France for the first time, 14 months previously. *E. histolytica* cysts were found in his stools. He underwent treatment with alcresta, and has remained negative after treatment now for over four weeks.

Case 11. He was taken ill with dysentery at La Bassée, France, in March, 1916. But he says that he first contracted dysentery in South Africa, twelve years

ago, and has never been really free from it since, passing blood and suffering from severe attacks of diarrhoea at intervals during these twelve years. *E. histolytica* cysts were found in his stools and he is now under observation after treatment.

Case 12. Developed dysentery at the Somme, 28th August, 1916. He was invalided to England. *E. histolytica* cysts were found in his stools. Alcresta was administered and he was discharged as cured on the 3rd January, 1917. He had never been out of England before going to France with the army.

Case 13. Contracted dysentery at Gallipoli in October, 1915. Relapsed in France in October, 1916, and was invalided to England. Cysts of *E. histolytica* were detected by us in his stools.

Case 14. This man first had dysentery at Guillemont, France, on the 30th September, 1916. He was invalided to England where *E. histolytica* cysts were found in his stools.

Case 15. He had dysentery for the first time at Beaumont Hamel, France, on the 31st December, 1916, after he had been three months out in France. He had never been abroad before. The cysts of *E. histolytica* were found in his stools. He is at present under observation after treatment.

Case 16. Developed dysentery at the Somme, November 26th 1916. His stools were found to contain *E. histolytica.* He was treated with alcresta and is still under treatment. He spent some years in New York; otherwise he had not been out of England before going to France with the army.

Case 17. This case had diarrhoea developing into dysentery, when he had been 17 months in France, and was at Abbéville, 20th December, 1916. He had never been out of England before. He was invalided home, and cysts of *E. histolytica* were discovered in his stools. He was treated with alcresta and he is still under observation.

Case 18*. Invalided home from France with pneumonia, December, 1916. He had been in France for one year and nine months, and had not suffered at all from diarrhoea or dysentery, either there or at any time in his life. He was found to be passing cysts of *E. histolytica* in his stools, and was treated with emetine bismuth iodide. During the war he had never been on any but the French front, but he says as a merchant sailor, before the war, he was on the River Plate once for three months.

Case 19. Contracted dysentery in France on the 1st December, 1916. He had previously seen service in the Dardanelles and Egypt, besides having served in the South African War. We found that he was passing the cysts of *E. histolytica* in his stools.

Case 20. Invalided home from France with persistent diarrhoea, January, 1917. Had been in France four months. He was billeted along with men returned from Gallipoli and Egypt. On examination of his stools in April, 1917, he was found to be passing cysts of *E. histolytica*, and was treated with emetine bismuth iodide. He had never been out of England before going to the French front in September, 1916.

SUMMARY

1. Twenty men, invalided from France, were found to be carriers of *E. histolytica*.

2. Eleven of these men had never been out of England before. One had been in Belgium before the war. Six had been in regions where amoebic dysentery is endemic, either during the war or earlier in their lives. Two were not questioned.

3. Four of the twenty had no history of dysentery, and declared that they had never had diarrhoea at any time.

Two who had no history of dysentery, said that they had suffered from 'very bad diarrhoea' in France for the first time in their lives; one of these men had been ill for a fortnight.

The other fourteen all had a history of dysentery.

4. Twelve of those with a history of dysentery had contracted it in France, ten of them in the Somme neighbourhood. (One of the eleven came invalided from Salonika, but his papers state that his illness there was a relapse from dysentery contracted previously in France.)

Two men had had dysentery before going to France, and their attacks of dysentery there were relapses. One of these had dysentery first in Gallipoli; the other had first had dysentery in South Africa twelve years before, and had been subject to attacks ever since.

It is cases like these last two which are most obviously responsible for the introduction of amoebic dysentery into the French trenches, although no doubt the 'non-dysenteric' carrier also plays his part.

PART V.—TREATMENT OF *E. HISTOLYTICA* INFECTIONS

From the middle of June, 1916, until the end of February, 1917, 134 cases harbouring *E. histolytica* have received some form of emetine treatment at the Tropical School Auxiliary Military Hospital.

TREATMENTS WITH ALCRESTA IPECAC.

One hundred and twelve cases were treated for the first time with alcresta, but if we take as a standard for 'cure' a negative period of at least two weeks after treatment, 19 of our 112 cases must be ruled out. This gives us 93 first treatments. Thirty-six of these (37·5 per cent.) were unsuccessful, i.e. the patient continued to pass cysts during and after treatment, or else 'relapsed' after treatment stopped.

The 93 cases received, however, very various amounts of alcresta, as shown in the following table.[1]

TABLE XII

Number of cases treated	Grs. emetine	Cured	Not cured
12	5 to 7·5	5	7
29	10 to 18	19	10
29	20 to 25	21	8
17	27 to 30	12	5
6	over 30	0	6

The six cases remaining uncured by 30 grs. emetine in the form of alcresta eventually proved to be resistant to various other forms of emetine treatment. Hence we cannot conclude that in the average case 30 grs. is likely to be less effective than 7.

Our general conclusions, then, from the use of alcresta ipecac. on 93 cases are:—

(1) A sufficient first treatment will cure about 65 per cent. of cases.

(2) A course of 20 to 25 grs. emetine in this form seems on the whole to give the best results. Ten grs. or less is probably insufficient.

[1] The usual daily dose was 5 tablets T.D.S. (=1·5 grs. emetine).

TREATMENT WITH METHYL EMETINE

Of six cases treated with methyl emetine only one was cured. (1 gr. was given daily for 15 days—in one case for 20 days.)

Methyl emetine has not so far had a very fair trial. There was only a small quantity of the drug available, and six cases is too small a number from which to draw any general conclusions. Of these six cases, only two were new cases; it was one of these that was cured. The other four showed themselves 'refractory' to previous treatment with alcresta and to subsequent treatment with emetine bismuth iodide, and appeared to be unusually resistant to emetine in any form. This is unfortunate, since it has weighted the chances against the success of methyl emetine so heavily. One can say nothing, therefore, as to the value of methyl emetine as a primary treatment.

TREATMENT WITH EMETINE BISMUTH IODIDE

1. *As a primary treatment*

One 3 grain tablet (containing about 1 gr. emetine) was given nightly.

Twenty cases were given as a primary treatment 15 to 60 grs. emetine bismuth iodide (= 5 to 20 grs. emetine). Two of them relapsed. One of the relapsing cases had had only 15 grs. of the biniodide, and was probably insufficiently treated.

2. *As a secondary treatment, after alcresta or methyl emetine*

(*a*) *After alcresta.* Seventeen cases that had resisted one, two, or more treatments with alcresta were treated with the biniodide. Six relapsed; two of them had had only 15 grs., but one had had 60 grs.

Of these six relapsing cases, a second course of the biniodide (30 to 60 grs.) so far has cured three. Two relapsed again.

(*b*) *After methyl emetine.* One case that had relapsed after methyl emetine was cured by a course of 15 grs. emetine bismuth iodide.

3. *As a third form of treatment, after alcresta and methyl emetine*

Three cases that had proved resistant to both alcresta and methyl emetine were treated with emetine bismuth iodide.

One of these cases had resisted three very long courses of alcresta

and two courses of methyl emetine. The others had resisted 24 grs. and 37·5 grs. emetine respectively in the form of alcresta, and a course of methyl emetine had also failed to cure.

On two of these three cases a course of 30 to 45 grs. biniodide produced no more than a temporary impression. They both relapsed again. The third case has had three successive courses of 15, 36, and 60 grs. biniodide, and has not yet been cleared up. The 60 gr. course seemed to have the least effect, cysts being passed throughout treatment.

From our short experience with emetine bismuth iodide, we find:—

(1) That out of 20 cases, only two relapsed after a first treatment.

(2) Treatment with emetine bismuth iodide has cleared up 14 cases out of 17 that had resisted alcresta only, and one that had resisted methyl emetine only.

(3) Three cases that had proved resistant both to alcresta and to methyl emetine have also proved resistant to emetine bismuth iodide.

PROVISIONAL COMPARISON OF THE RESULTS OBTAINED WITH TWO FORMS OF EMETINE

If we eliminate from our total of 93 alcresta cases and 20 biniodide cases all those having less than 10 grs. emetine in their first treatment we find that:—

81 first treatments with alcresta were followed by 29 failures to cure (= 35·8 per cent.).

16 first treatments with biniodide were followed by 1 failure to cure (= 6·2 per cent.).

It is impossible to make close comparison between numbers so unequal in the two groups, but the greater efficacy of the biniodide is certainly indicated.[1]

It is further indicated by the fact that of 17 cases relapsing after alcresta 14 were cured by the other treatment.

On the other hand there seems to be, in any sufficiently large group of cases, a certain residue which remains quite unaffected by emetine bismuth iodide, as well as by alcresta and methyl emetine.

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1 Dobell (1917) gives 11 per cent. of relapses as the most unfavourable estimate in 24 cases treated with 26 grs. and over (8·6 grs. emetine and over.)

DATE OF RELAPSE AFTER TREATMENT

In our previous paper we pointed out that, when relapses occur after treatment, they occur chiefly in the first week. We considered two weeks as the *minimum* period over which post-treatment observation should extend; most of our cases have been observed for longer.

If now we consider our total up to the end of February of 134 cases, and deduct from these the 19 that were not observed long enough, we have 115 cases which received, in all, 166 treatments. In 14 instances the cysts did not disappear during treatment. Fifty-two of the remaining 152 relapsed after treatment. The incidence of these relapses is as follows:—

Of 152, negative at the end of treatment,	32 relapsed in the 1st week.
Of 120, hitherto negative,	10 relapsed in the 2nd week.
Of 67, hitherto negative,	3 relapsed in the 3rd week.
Of 42, hitherto negative,	5 relapsed in the 4th week.
Of 19, hitherto negative,	2 relapsed in the 5th week.

It is true that some of the cases which were followed for such long periods as four to five weeks were such as had already relapsed once or twice, and so the later groups were in some degree selected material. But this does not apply to all the late relapses. One case relapsed for the first time on the twenty-fourth day, one on the twenty-fifth, and one on the thirty-third day after the end of treatment. Considering the steadily diminishing numbers of cases examined in the fourth and fifth weeks, the occurrence of even three such late relapses is rather disquieting.

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THE OCCURRENCE OF *ANKYLOSTOMA CEYLANICUM* IN WEST AFRICAN DOGS

BY

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AND

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In a paper (1915) entitled 'Ankylostomiasis in Dogs in Sierra Leone,' we drew attention to the existence of two species of ankylostoma (*A. caninum* and *A. ceylanicum*) in all of seven native dogs examined by us in Freetown. The following is an extract from this paper:—

'These species are readily distinguished one from the other by the characteristic arrangement of their teeth. The mouth of *Ankylostoma caninum* is armed with three pairs of prominent ventral teeth, whereas in *Ankylostoma ceylanicum* there is one pair of large ventral teeth, and one very small pair near the base of the former, but on a slightly deeper plane. These characters are illustrated in figs. 1 and 2.

'The bursa of the males is very similar in the two species. It consists of two large lateral and a small dorsal lobe. The arrangement of the rays is as follows:— In each lateral lobe there is an anterior ray which is cleft, an antero-external ray, a median ray which is doubled, and a postero-external ray which arises from a common trunk with the single posterior ray. In the dorsal lobe is the posterior ray, which exhibits slight differences in the two species. In both it is bifurcated in its terminal third, and each of the branches is at its extremity tridigitate. It is in the character of these terminal digitations that the slight difference is found (see figs. 3 and 4). In both species the two inner digitations are small, being separated by a mere notch. In *Ankylostoma caninum* the cleft separating the two inner from the outer digits is shallow, but in *Ankylostoma ceylanicum* the cleft is deep, being about half the length of the branch of the posterior ray.'

Leiper (1915) in criticism of this paper makes the following observations:—

'The details given in this paper lead me to doubt the accuracy of the diagnosis of *A. ceylanicum*. The authors compare their presumed *A. ceylanicum* with *A. caninum* and illustrate the main points of contrast with figures reproduced here. . . . . .

'I append a drawing (fig. 7) from an Indian specimen of *A. ceylanicum*; it will be seen that the dorsal ray has a pair of digitations only on each of its two branches. As this division is of specific importance and occurs in all specimens, it seems unlikely that the West African dog ankylostome is the same species as that recorded above. The drawings of the mouth capsule given by Yorke and Blacklock certainly show a single pair of large chitinous teeth as in *A. ceylanicum*, but the outline is scarcely correct. One is inclined to think that these authors have been dealing with *Uncinaria stenocephala* Raillet, a similar ankylostome often found in association with *A. caninum*.

'As regards synonomy a recent article by Gomes de Faria contributes further anatomical details which tend to show that *A. braziliense* and *A. ceylanicum* are distinct species.

'Some attention has already been devoted to ankylostomiasis in Sierra Leone. Major F. Smith in a paper in the Journal of the Royal Army Medical Corps for 1905 says: "*I have not yet found in Sierra Leone a dog free from ankylostomes*," but he does not indicate that man and the dog in those regions have a species in common.'

We propose here to discuss the various points which are raised.

I.—'*The drawings of the mouth capsule given by Yorke and Blacklock certainly show a single pair of large chitinous teeth, as in* A. ceylanicum, *but the outline is scarcely correct. One is inclined to think that the authors have been dealing with* Uncinaria stenocephala (*Raillet*), *a similar ankylostome often found in association with* A. caninum.'

In reply to this we may state at once that the mouth capsule of the ankylostome which we found in dogs in Sierra Leone bears no resemblance to that of *Uncinaria stenocephala*. As shown in the figure given in our paper, the Sierra Leone ankylostome has 'one pair of large ventral teeth, and one very small pair near the base of the former, but on a slightly deeper plane.' So different is the appearance of the mouth capsule of *Uncinaria stenocephala*, which has a pair of large cutting plates in no way resembling the ventral teeth of the ankylostome in question, that it did not occur to us that any confusion with this species could arise. We do not

know upon what evidence Leiper made the assertion that the outline is scarcely correct, as he has not seen the specimens from which the diagram was made and our description framed.

II.—'*I append a drawing from an Indian specimen of* A. ceylanicum. *It will be seen that the dorsal ray has a pair of digitations only on each of its two branches. As this division is of specific importance and occurs in all specimens, it seems unlikely that the West African dog ankylostome is the same species as that recorded above.*'

With regard to the termination of the dorsal ray, we might point out that it is commonly held that in the genus Ankylostoma each of the primary branches into which the dorsal ray divides is at its extremity tridigitate. Raillet and Henry (1909) define in the following manner the group Ankylostomeae:—'Bourse caudale à côtes antérieures fendues, moyennes dédoublées, postérieures et postérieures externes naissant d'un tronc commun, postérieures tridigitées.' Clayton Lane (1916), writing of the genus Ankylostoma, states: 'The dorsal ray bifurcates, each branch further bifurcating, while the inner of these sub-branches again ends in two points.'

In the plate which illustrates his paper, Lane gives two figures of the termination of the dorsal ray of *A. ceylanicum*. This is shown to be bifurcated and each of the branches is at its extremity tridigitate: the two inner digits are small, being separated by a notch. These figures, which were published by Lane nearly a year after our paper appeared, resemble that given by us very closely.

Looss (1911), in his original description of *A. ceylanicum*, gives no detailed account of the termination of the posterior ray of the bursa. In a letter published by de Faria,* Looss writes: 'The relative thickness of the bursal rays in similar species of ankylostoma is a definite differential character, but this point is emphasised by no recent writer. On the other hand one finds full descriptions of the arrangement of the rays, which is the same for all ankylostoma, and complete details of the terminal divisions of the dorsal ray, which vary in nearly every individual.'

* Quoted by Clayton Lane, loc. cit.

It is very instructive to compare two drawings by Leiper of the dorsal ray of *A. ceylanicum*, the first in the *Journ. of Trop. Med. and Hyg.*, 1913, Vol. XVI, p. 335, and the second in the *Journ. R.A.M.C.*, 1915, Vol. XXIV, p. 572. When Leiper wishes to prove that Lane's *A. ceylanicum* of India is the same as de Faria's *A. braziliense*, he figures a notched inner sub-branch, whereas when he desires to show that the ankylostome from Sierra Leone described by us as *A. ceylanicum* is not identical with the Indian *A. ceylanicum* he omits the notch.

As will be seen from the extract quoted above, we did not in our paper attach the same diagnostic significance to the character of the posterior ray as does Leiper. Looss gave no illustration of the termination of the posterior ray in his original description of *A. ceylanicum*, and consequently we were compelled, in identifying the West African worm, to rely mainly on the characteristic arrangement of the ventral teeth. Recently, as already mentioned, Lane has furnished drawings of the dorsal ray of the Indian *A. ceylanicum*, and we have had an opportunity of examining a specimen of this worm. On comparing the Indian specimen with those from West Africa slight differences in the posterior ray are observable—the ray in the West African worm is a little thicker and the notch in the sub-branch is a trifle deeper. Whether these slight differences are constant, or what importance should be attributed to them we do not know. In this connection we might recall the following statement by Looss in his original description of *A. ceylanicum*: 'All the rays are remarkably thick and plump.'

III.—*Major F. Smith, in a paper in the Journal of the Royal Army Medical Corps for* 1905, *says*: *'I have not yet found in Sierra Leone a dog free from Ankylostomes'; but he does not indicate that man and the dog in these regions have a species in common.*

This appears to be used by Leiper as an argument against our suggestion that the parasite found by us in dogs may also occur in man. As Major Smith does not give any details by which the parasites he found in dogs can be recognised, reference to his work seems hardly relevant. However, it is interesting to note that Major Smith, a few lines below that quoted by Leiper, writes: 'The experimental results suggest intercommunicability among animals;

inferentially we may suspect that man can contract ankylostomiasis from the lower animals.'

For purpose of comparison we give here camera lucida drawings of the outline of the mouth parts and dorsal ray of the bursa of *A. ceylanicum* from West African dogs, *A. ceylanicum* from India (sent by Dr. Clayton Lane), and *Uncinaria criniformis* (*stenocephala*) (from Looss). These drawings were made by Mr. Forster Cooper, to whom we are much indebted.

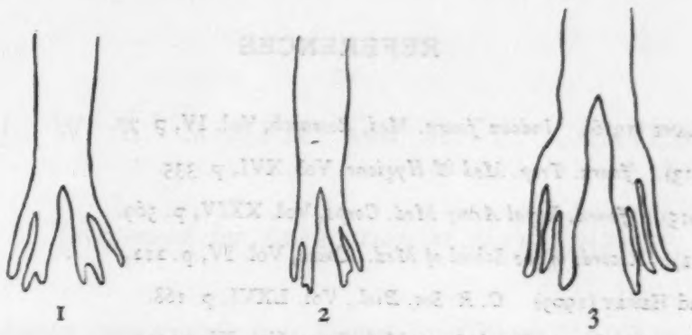


Fig. 1. Dorsal ray of *A. ceylanicum* (West African dog).
Fig. 2. Dorsal ray of *A. ceylanicum* (India).
Fig. 3. Dorsal ray of *U. criniformis*.

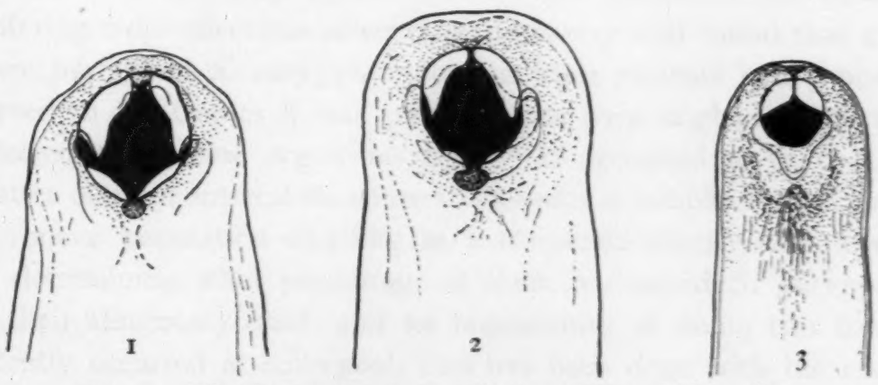


Fig. 1. Mouth capsule of *A. ceylanicum* (West African dog).
Fig. 2. Mouth capsule of *A. ceylanicum* (India).
Fig. 3. Mouth capsule of *U. criniformis*.

Dr. Macfie informs us that he has found *A. ceylanicum* in four of ten dogs examined by him in Accra, and that he has recorded the observation in his Annual Report to the Colonial Office (1916) now in the press.

Leiper (1913) wrote: 'I have re-examined my collections of ankylostoma from cats and dogs, mainly from Africa, but this species [*A. ceylanicum*] is not represented.' Possibly it was this fact which called forth the unwarrantable criticism of our paper.

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THE OCCURRENCE OF *SPIROCHAETA EURYGYRATA* IN EUROPEANS IN ENGLAND WITH A NOTE ON A SECOND SPECIES OF *SPIROCHAETA* FROM THE HUMAN INTESTINE

BY

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(*Received for publication* 21 *May*, 1917)

Spirochaeta eurygyrata was found by Carter (1916) in the stools of 56·5 per cent. of the dysenteric patients from Gallipoli and Egypt examined by him, and, in West Africa, Macfie (1917) recorded that it was 'well-nigh an invariable inhabitant of the human intestine at Accra on the Gold Coast.' Carter also examined 100 patients suffering from affections other than dysentery and found that 41 of them harboured *S. eurygyrata*, but as these patients had probably served in the tropics it was possible that they might have become infected with these organisms there. It appeared to us to be a matter of some interest therefore to examine a sample of an average European population dwelling in a temperate climate with a view to determining what percentage of them harboured *S. eurygyrata* in their alimentary tract, and an opportunity of doing this having recently occurred at Liverpool, this has been done with the results recorded below.

Altogether 105 persons were examined, namely, 82 hospital patients (59 men and 23 women) and 23 normal men. The hospital patients were all suffering from some surgical condition but not from any intestinal disorder; the normal men were young, healthy individuals. None of the patients, so far as could be ascertained, had ever resided in the tropics.

In each case only a single smear was examined, so that the

proportion of positive findings is probably considerably below the actual percentage. The normal men and the majority of the hospital patients were only examined once. Nineteen of the hospital patients were examined on two occasions or more often, but only three of these were negative at the first but positive at one of the later examinations.

Of the 105 patients examined 59, or 56·2 per cent., were found to harbour *S. eurygyrata* (see Table); but a larger proportion of the hospital patients (59·8 per cent.) was infected than of the normal men (43·8 per cent.). Why this should have been the case cannot definitely be explained, but it may be pointed out as a possible cause that the normal men were all quite young fellows in excellent physical condition, whereas the hospital patients were on the average older and less robust. It is possible, however, that the care taken in hospital to keep the bowels active may have had something to do with the results, as it has been noted that spirochaetes are most readily found in loose motions.

TABLE

	Number examined	Number harbouring *S. eurygyrata*	Percentage
Hospital Patients	82	49	59·8
Normal Men	23	10	43·8
Totals	105	59	56·2

The proportion of these hospital patients found to harbour *S. eurygyrata* (59·8 per cent.) was very similar to that found by Carter amongst his dysentery cases (56·5 per cent.) but greater than that found in his patients suffering from other conditions (41 per cent.). In the latter the proportion was similar to that found in the healthy normal men (43·8 per cent.), a point of some interest as indicating perhaps that this is the usual proportion in young adult men.

In the majority of the cases the spirochaetes were far from common, and in none of them were the infections so massive as many of those seen by one of us [J. W. S. M.] in West Africa.

From the examination of this small sample of an average European population it would appear therefore that *S. eurygyrata* is a frequent inmate of the human intestine in temperate climates, and may be found in over 50 per cent. of individuals.

A SECOND SPECIES OF *SPIROCHAETA* FROM THE HUMAN INTESTINE

In the course of making the examinations for *S eurygyrata*, of which a brief note is given above, a second type of spirochaete was met with on one occasion. It is greatly to be regretted that the organisms were only discovered in a dried film which had been treated with gentian violet, and that therefore their cytology could not be studied after suitable fixation and staining. Nevertheless as they were clearly different from *S. eurygyrata*, the common spirochaete of the human intestine, and as they may be met with by others in the examination of faeces, a brief note on the features that we were able to make out may not be out of place.

The patient in which this organism was found was an adult European woman, who was in hospital having undergone the operation of gastro-enterostomy.

The spirochaetes, which were but scanty in the faeces, were much larger organisms than *S. eurygyrata*. Twenty individuals taken as they came were drawn with the aid of a camera lucida, and measured by the compass method. Of these the shortest measured 9μ, the longest 23μ, and the average length worked out at 16·2μ. The spirochaetes were about 0·3μ to 0·4μ in thickness, and their ends tapered slightly but were rather blunt. In some specimens definite bars suggesting a chambered structure could be made out, and in others rounded masses resembling coccoid bodies were present. One or two of the organisms showed an appearance that might have been due to the presence of an undulating membrane. The body of the spirochaetes was bent into a number of shallow and rather irregular waves (see figs. 1 and 2).

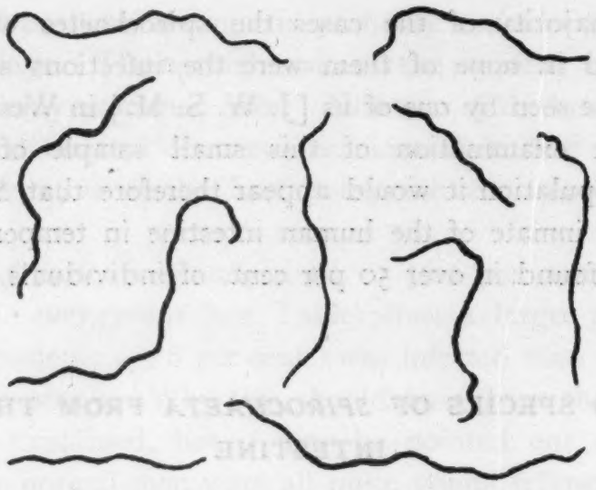


FIG. 1. A large coprophytic spirochaete found in a European. × 2000.

Unfortunately the spirochaetes were not observed alive in this case, but in a previous patient examined by one of us [H. F. C.] similar organisms were seen which were actively motile and were a conspicuous feature in the fresh preparations. These spirochaetes in stained films have been re-examined, and have been compared with those found in the more recent case and appear to be identical. In two other cases of our series we have found bodies which may have been specimens of the same spirochaete, but they were so very rare in the films that we cannot be certain on this point.

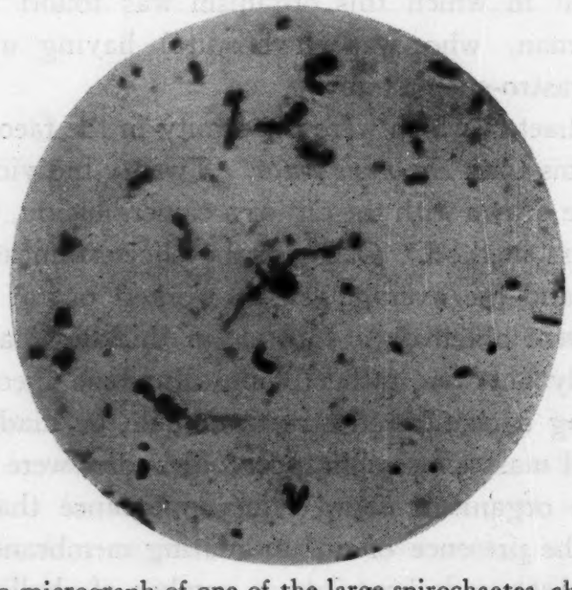


FIG. 2. Photo-micrograph of one of the large spirochaetes, about 18μ long, found in a European.

J. G. and D. Thomson (1914) refer to a spirochaete they found in a case of amoebic dysentery which was much larger than *S. eurygyrata*, varied in length from 8μ to 20μ, had irregular coils and rather blunted ends. This description, and the figures accompanying it, corresponds with that given by us of the spirochaete we have found, and it is possible that it may refer to the same organism. These authors, however, add that this spirochaete 'seems to correspond to a certain extent to the type described by Le Dantec.' We have carefully considered the account of the spirochaetes found in cases of dysentery by Le Dantec (1903), and we are convinced that it refers to an organism differing substantially from the large spirochaete we have described. The size of the spirochaete, 6μ to 14μ, suggests rather that he was dealing with *S. eurygyrata*.

With the doubtful exception of the organism referred to by J. G. and D. Thomson, we have been unable to trace any description of a large intestinal spirochaete of man similar to that found in our patient, and we therefore propose the name *Spirochaeta intestinalis* for this organism should it prove to be a new species.

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THE RELAPSING FEVER SPIROCHAETES

BY

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AND

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The spirochaetes found in the blood in relapsing fever in different parts of the world have been separated both by morphological features and biological reactions into a number of so-called species, but so far as morphology is concerned there appears to be great dissimilarity between the descriptions of different authors when dealing with the same organism.

The species most generally recognised are *S. recurrentis*, *S. carteri*, *S. novyi*, and *S. duttoni*, the organisms found in the relapsing fevers of Europe, Asia, America, and Africa respectively. Novy and Knapp (1906) appear to be mainly responsible for considering as distinct species the spirochaetes found in American, Indian, and African relapsing fever. They give a table in which the distinctive morphological characters are set forth. The length of the American and Indian spirochaetes is stated to be 8μ to 20μ, whereas that of the African is 16μ to 30μ. The other differential points mentioned relate to the width of the filament, the number of turns, the distance of the turns, and the width of the turns or spirals. Many of the modern text-books of Tropical Medicine appear to have followed Novy and Knapp. It has, however, been suggested that the spirochaetes of all the above infections may be varieties of a common type transmitted by different arthropods, and Nuttall (1912) writes that *S. recurrentis* may be the only true species and that the various specific names given to the spirochaetes causing relapsing fever in man, although convenient to distinguish strains or races of different origin, 'cannot be regarded as valid names, in the sense of scientific nomenclature, for virulence and immunity reactions are not adequate tests of specificity.'

An opportunity having occurred of examining the spirochaetes

of European, Indian, and African relapsing fever, it was decided to study the variation in length of these parasites by the method employed previously in the case of *S. eurygyrata* and other spirochaetes (Macfie, 1916). The materials used were in the case of Indian relapsing fever and *S. duttoni* specimens preserved in the collection at the Liverpool School of Tropical Medicine, and in the case of European relapsing fever blood films made in 1915 from two patients infected in the Balkans. All the specimens were dried blood films, fixed with absolute alcohol, and stained by the Romanowsky method.

In order to determine the variations in length of these spirochaetes, eight blood films showing *S. duttoni*, eight showing *S. carteri*, and four showing *S. recurrentis* were selected, and in each of these twenty-five spirochaetes, taken as they came, were drawn with the aid of a camera lucida and measured by the compass method. In all the blood films more or less coiled or twisted spirochaetes were found, and others that showed very slight undulations, so that any measurements taken across the waves would have been quite unreliable.

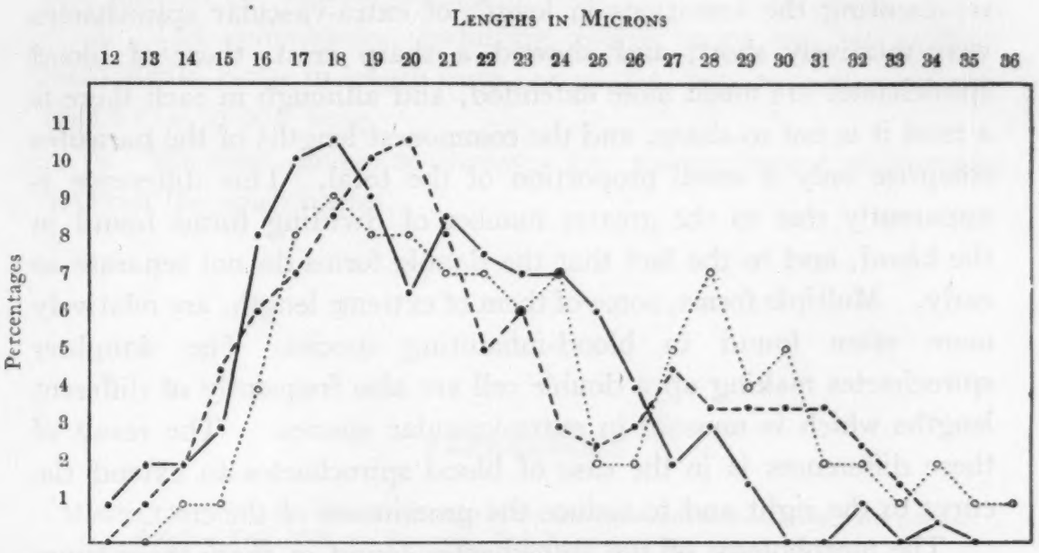
In this way 200 specimens of *S. duttoni*, 200 of *S. carteri*, and 100 of *S. recurrentis* were measured (see Table). *S. duttoni* varied

The distribution according to length, by percentages, of Relapsing Fever spirochaetes.

Type of Relapsing Fever	Number of spirochaetes measured	Length in Microns												
		12	13	14	15	16	17	18	19	20	21	22	23	24
African, *S. duttoni* ...	200	1	2	2	3	**8**	**10**	**10·5**	**9**	6·5	8·5	7·5	7	7
Indian, *S. carteri* ...	200	...	1	2	4·5	6	7	**8·5**	**10**	**10·5**	**8**	5	6	3
European, *S. recurrentis*	100	...	...	1	1	4	**8**	**9**	**8**	**8**	7	7	6	7

Type of Relapsing Fever	Number of spirochaetes measured	Length in Microns												Average length
		25	26	27	28	29	30	31	32	33	34	35	36	
African, *S. duttoni* ...	200	6	4	2	3	1·5	...	...	1	...	0·5	...	...	20·56
Indian, *S. carteri* ...	200	2·5	3	4·5	3·5	3·5	3·5	3·5	2·5	1·5	0·5	...	...	21·86
European, *S. recurrentis*	100	2	2	5	7	4	5	2	2	1	2	1	1	23·07

in length from 12μ to 34μ, average 20·56μ; the biometric curve was low and extended, rising to a peak at 18μ, with a secondary crest at 21μ (see Graph), and showing the commonest lengths of the



Graph showing the variations according to lengths, by percentages, of *S. duttoni* (●—●—●—), *S. carteri* (● - - ● - - ● -), and *S. recurrentis* (o·····o·····o··).

spirochaetes to be 16μ to 19μ. *S. carteri* varied in length from 13μ to 34μ, average 21·86μ; the curve showed a peak at 20μ, a subsidiary crest at 23μ, and the commonest lengths of the spirochaetes were 18μ to 21μ. *S. recurrentis* ranged from 14μ to 36μ in length, average 23·07μ; a large number of double forms had been present in the blood at the time when the films were made, and these, together with the fact that only a small number of parasites were measured, made the curve an irregular one. The commonest lengths of the spirochaetes were, however, 17μ to 20μ.

Comparing these measurements, it will be seen that the differences between these three types of spirochaetes are but slight. The range of length is practically the same, the groups comprising the commonest lengths overlap, and the crests of the curves formed by distributing the parasites according to length occur close together. These observations show that *S. duttoni*, *S. carteri* and *S. recurrentis* do not differ appreciably as regards length.

There is a very marked difference between blood spirochaetes and the extra-vascular species previously measured (Macfie, 1916) as

regards variations in length. This difference is apparent even without measurements, but is most clearly seen in biometric curves. A similar difference has also been observed in the case of *S. marchouxi* (Annual Report of the Accra Laboratory, 1916). The curves representing the variations in length of extra-vascular spirochaetes were relatively short, and showed a sharp crest, those of blood spirochaetes are much more extended, and although in each there is a crest it is not so sharp, and the commonest lengths of the parasites comprise only a small proportion of the total. This difference is apparently due to the greater number of dividing forms found in the blood, and to the fact that the double forms do not separate so early. Multiple forms, some of them of extreme length, are relatively more often found in blood-inhabiting species. The daughter spirochaetes making up a double cell are also frequently of different lengths which is unusual in extra-vascular species. The result of these differences is in the case of blood spirochaetes to extend the curve to the right and to reduce the prominence of the crest.

The morphology of the spirochaetes found in these three types of relapsing fever was similar also in other respects. The thickness of the organisms in these dried blood films was about the same, namely, approximately 0·3μ; the ends were pointed; unstained bars or gaps occurred in the bodies of all three types; and no undulating membrane was ever observed. The terminal flagellum described by some writers was not seen, but at each end of the spirochaetes there was as a rule a part of the body about one micron long which stained more palely than the rest of the parasite.

Much importance has been attached to the number and size of the undulations of these spirochaetes, but so far as our observations go no difference could be made out in this respect between the European, Asian, and African strains. All three showed wide undulations that varied greatly both in size and number, some spirochaetes being extended as almost straight lines, others coiled into rings, but perhaps the commonest wave length was about 4μ and amplitude about 0·8μ in all three strains. In the slides from cases of European relapsing fever a few closely coiled individuals were found similar to those described by Novy and Knapp in *S. novyi*; the wave length in these specimens was about 2·2μ, and the amplitude of the wave about 0·5μ.

SUMMARY

1. There is no appreciable difference in length between the spirochaetes causing European (*S. recurrentis*), Indian (*S. carteri*), and African (*S. duttoni*) relapsing fever.

2. The spirochaetes, including double forms but not multiple individuals, range in length from 12μ to 36μ, but are most commonly about 17μ to 20μ long.

3. We were unable to discover any morphological distinctions between the spirochaetes.

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PERSONS WHO HAVE NEVER BEEN OUT OF GREAT BRITAIN AS CARRIERS OF *ENTAMOEBA HISTOLYTICA*

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In this preliminary note we record the result of examining for intestinal protozoa 344 persons who have never been out of Great Britain.

More than half of these (206) were healthy young men of about 18 years of age who had recently entered the army and were in training at a camp in the vicinity of Liverpool. A single examination revealed the following infections:—

E. histolytica cysts	8 =	3·9 per cent.
E. coli cysts	22 =	10·7 ,,
Giardia intestinalis	9 =	4·3 ,,

The remaining cases (138) were patients, for the most part surgical, in the Liverpool Royal Infirmary; they consisted of both males and females of various ages. A single examination showed the following infections:—

E. histolytica cysts	2 =	1·4 per cent.
E. coli cysts	4 =	2·8 ,,
Giardia intestinalis	5 =	3·6 ,,
Tetramitus mesnili	2 =	1·4 ,,

Of the two cases infected with the cysts of *E. histolytica*, one was a boy of 14 and the other a young man aged 24.

In the following table various details are given regarding the ten cases infected with *E. histolytica* cysts; none of them gave a history of dysentery.

Number of Case	Age in years	Place of residence	Civil occupation	Date of joining Army and going to training camp
1	18⅓	Manchester	Clerk	March 12, 1917
2	18⅓	Ashton-under-Lyne ...	Spinner	March 7, 1917
3	18¼	Chorley	Bleaching Works Employee	March 14, 1917
4	18 5/12	Ardwick	Carter	March 7, 1917
5	18½	Liverpool	Dock Labourer ...	March 12, 1917
6	18¾	Wigan	Carter	March 5, 1917
7	18	Burnley	Shop Assistant ...	March 12, 1917
8	18⅓	Manchester	Packer	March 12, 1917
9	14	Liverpool	School Boy ...	—
10	24	St. Helens	Chemical Works Employee ...	—

Having satisfied ourselves by careful study of fresh and stained preparations that the cysts found in the faeces of these ten cases were indistinguishable morphologically from those present in the faeces of convalescent dysenterics, we decided to examine their pathogenicity. With this object in view, four kittens (Nos. 31-34) were fed on portions of the infected faeces from Cases 3, 1, 2 and 7 respectively. A few amoebae were found in the faeces of one of the animals (Kitten 32) six days after the infective feed; on the twelfth day symptoms of acute dysentery developed, the animal passing mucus, blood epithelium and many amoebae. Acute dysenteric symptoms persisted to the death of the animal, which occurred on the twenty-third day. During the whole of this time large numbers of amoebae were passed with the faeces. Neither in the fresh nor in stained preparations could these be distinguished from those found in the stools of human beings suffering from acute amoebic dysentery.

On post-mortem examination of the kitten a large portion of the

mucosa of the large intestine appeared to be superficially necrosed. In scrapings, enormous numbers of amoebae were found. Scattered throughout the large intestine were small discrete ulcers. Sections for microscopic examination are in the course of preparation. Bacteriological examination of the faeces of Case 1 on which the kitten was fed, and also of the kitten itself during the period of acute dysentery, and of a scraping of the large intestine, were made by Professor Glynn. No bacilli of the dysenteric group were found.

Kitten 31 died six days after the infective feed. No amoebae were found in its faeces; there were no dysenteric symptoms, nor was any lesion of the large intestine found on post-mortem examination. Kittens 33 and 24 are alive and well at the time of writing, eighteen days after the infective feed.

The successful infection of Kitten 32 establishes the fact that Case 1—a healthy youth of 18, who had never been out of England and Wales and who has never suffered from dysentery—is an amoebic dysentery carrier in as true a sense as convalescent dysenterics from the tropics or sub-tropics.

The observations recorded in this note are of considerable interest, especially in view of the fact that acute amoebic dysentery originating in Great Britain is almost unknown. So far as we are aware, only three definite cases have hitherto been recorded. In 1909, Saundby and Miller published an account of a case of acute amoebic dysentery with hepatic abscess in a man who had never been out of England. Marshall (1912) describes a case of amoebic dysentery in a man who had never been out of Scotland; while in 1916, Worster-Drought and Rosewarne give details of a second case of dysentery in a man who had never been out of England.

It should be noted that of the two groups into which the cases examined by us were divided—young recruits in a military camp and civilians in surgical wards of a civil hospital—the incidence of *E. histolytica* cysts was nearly three times greater in the former batch than in the latter. In the camp were also a number of men who had returned from the Mediterranean. When examined the young recruits had been in camp about two months.

At the present time we content ourselves with merely stating these details; as the work is being continued, we withhold further discussion for a future communication.

SUMMARY

Of 344 persons who had never been out of Great Britain at least ten (2·9 per cent.) harboured in their faeces cysts morphologically indistinguishable from those of *E. histolytica*. By feeding experiments on kittens the cysts in one of these cases were proved to be pathogenic.

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STUDIES IN THE TREATMENT OF MALARIA

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INTRODUCTION

To be efficient any mode of treatment of malaria must effect two objects, firstly the control of the acute attack and secondly the prevention of relapses.

Although quinine accomplishes the first of these objects, the ideal drug, which while causing the acute attack to subside also prevents the occurrence of relapses, is at present unknown, and hence further treatment with a view to preventing relapses is always necessary. In considering the records in the literature of the various treatments of the acute stage of the disease, we found a lack of precise information upon which to base a sound conclusion regarding their respective merits. For instance, quinine can be administered in various ways, e.g., orally, intramuscularly or intravenously; again it may be given before, during or after a paroxysm, and in various amounts which may be taken at a single dose or in a number of partial doses. How, when, and in what quantity should quinine be given are then questions to which, so far as we are aware, the literature affords no satisfactory

answer. As regards the prevention of relapses, the evidence advanced in favour of any of the multitude of methods recommended is even more defective. In lieu of ascertained facts, we have too frequently the mere opinions of those who in their day were hailed as 'authorities.' 'Like echoes that beget each other amongst the mountains, the praise or blame of such men rolls in volleys long after the report from the original blunderbuss.'

We have therefore set ourselves the task of obtaining definite information—clinical and microscopical—of the value of certain modes of treatment, and intend, so far as is feasible, so to conduct our inquiry as to render possible a comparison of their respective values. The ultimate aim of researches of this kind is of course the discovery of a curative mode of treatment. Though this object may not be attained, we hope to furnish detailed records of experimental work which will have a permanent value, as without such information further advance is almost impossible. Had such records previously existed we should have been saved the greater part of our preliminary work.

Proof of a cure is difficult to obtain; the absence of parasites from the blood is inconclusive, for such a condition frequently occurs in the intervals between the relapses. Whether the leucocyte formula or some other test will suffice to decide the point is a matter for further research; meanwhile, failing any criterion, we are left in the unfortunate position of waiting till the passage of time settles the question. In estimating the efficacy of a curative treatment, it is necessary to observe an adequate number of cases, for that spontaneous cure is effected in the course of time there can hardly be any doubt.

I. INTRAVENOUS INJECTIONS OF TARTAR EMETIC

Sir Leonard Rogers (1917), in a paper entitled 'Disappearance of Malignant Tertian Crescents from the Blood following the Intravenous Injection of Tartar Emetic,' gives the results obtained by himself in three malignant tertian (crescent) cases and two simple tertian cases, records two other simple tertian cases treated in

the same way by Capt. Hume, I.M.S., and concludes that 'the drug has a destructive effect on the gametes of the benign tertian as well as the malignant tertian variety of malaria, and makes it probable that it will act similarly on the quartan form.'

Before recording our own investigations on this mode of treatment, which in the opinion of Sir Ronald Ross (1917) 'promises to be one more important discovery to his [Rogers'] already distinguished account,' we must draw attention to the following statements made by the author in his paper.

1. '*Nevertheless, even quinine is not an ideal drug for the disease, because, although it rapidly kills the intracorpuscular stage of the parasite and brings about the cessation of the febrile paroxysms, yet it completely fails to destroy the extracorpuscular cycle, which is responsible both for the frequent relapses of the ague, and, still more important, for the infection of mosquitos, and through them of other persons.*' This sentence is not clear to us. Presumably, by the intracorpuscular stage is meant trophozoites and schizonts, whilst 'extracorpuscular cycle' means gametes. If this be so, then Rogers assumes without, so far as we are aware, any proof, that gametes differ from schizonts in being extracorpuscular. Further, he asserts as a fact that gametes are responsible for relapses; this is far from being proved.

2. '*It is well known that once they* [*gametes*] *appear in the blood they remain present for months on end quite uninfluenced by quinine.*' This is an astonishing statement. As regards crescents it is contrary to our experience and that of others. To quote two instances only:—

Thomson (1911) found that 'quinine reduces the crescents to numbers less than one per c.mm. of blood within three weeks, provided it be given in daily doses of twenty to thirty grains.'

Darling (1914) states 'it was found that with a daily dose of 10 grs. of quinine a gradual but steady decrease in the number of gametocytes and even of crescents took place.'

In so far as simple tertian gametes are concerned, the statement is also erroneous. In the eleven cases of simple tertian malaria recorded in this paper, the gametes, while unaffected by antimony, disappeared within two to three days after the administration of quinine.

These erroneous beliefs on the part of Rogers afford, in our opinion, the only excuse for his neglect to make control observations. If he had made a sufficient number of these he would have found, firstly, that gametes of both species may disappear at least temporarily from the peripheral blood without any treatment at all, and secondly that their disappearance is considerably accelerated by quinine. This is the experience of other observers; thus James (1912) states 'if quinine be exhibited in full doses the gametes [of tertian malaria], no matter how plentiful at first, will disappear in four or five days.' Again, Billet (1913), who confirms Rieux (1913), states 'Les éléments sexués ou gamètes au contraire résistent vingt quatre à quarante huit heures et quelquefois davantage à son action.'

Having drawn attention to some of the misleading assertions in this paper, we shall now consider the results obtained by us in the treatment of twenty-two cases of malaria by intravenous injection of tartar emetic. All the cases were adult male Europeans, and all had contracted the infection in Macedonia at least six months previously. The solution used for injection was 2 per cent. tartar emetic in water containing 0·5 per cent. phenol.

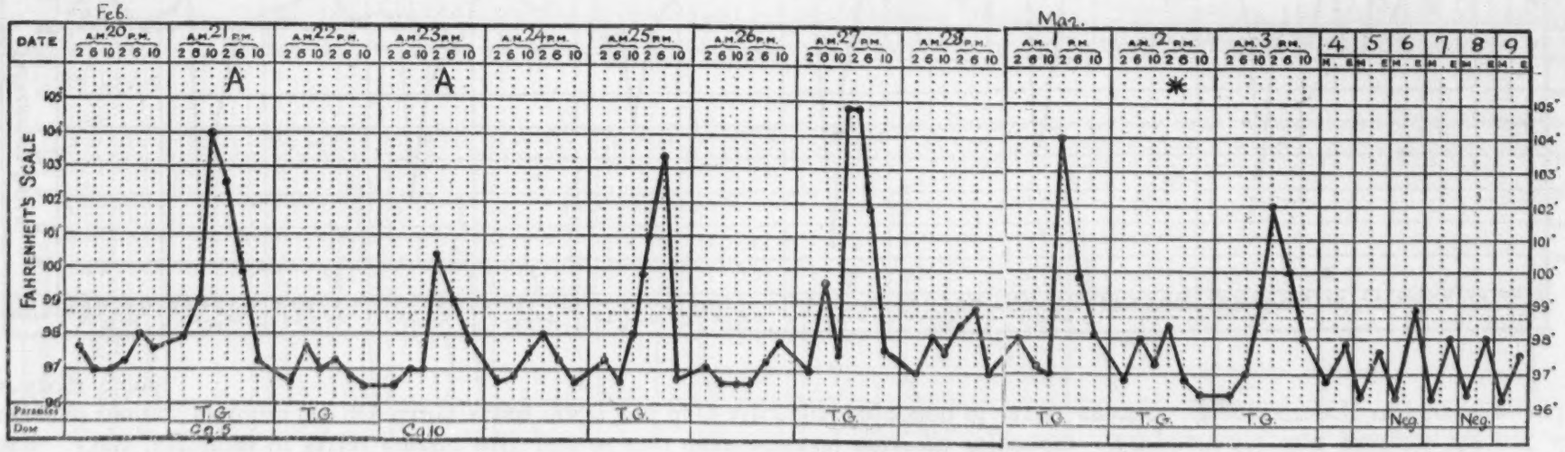
I. SIMPLE TERTIAN MALARIA (Cases 1—11)

Ten of these cases were treated with tartar emetic alone, and one (Case 11) with both tartar emetic and quinine.

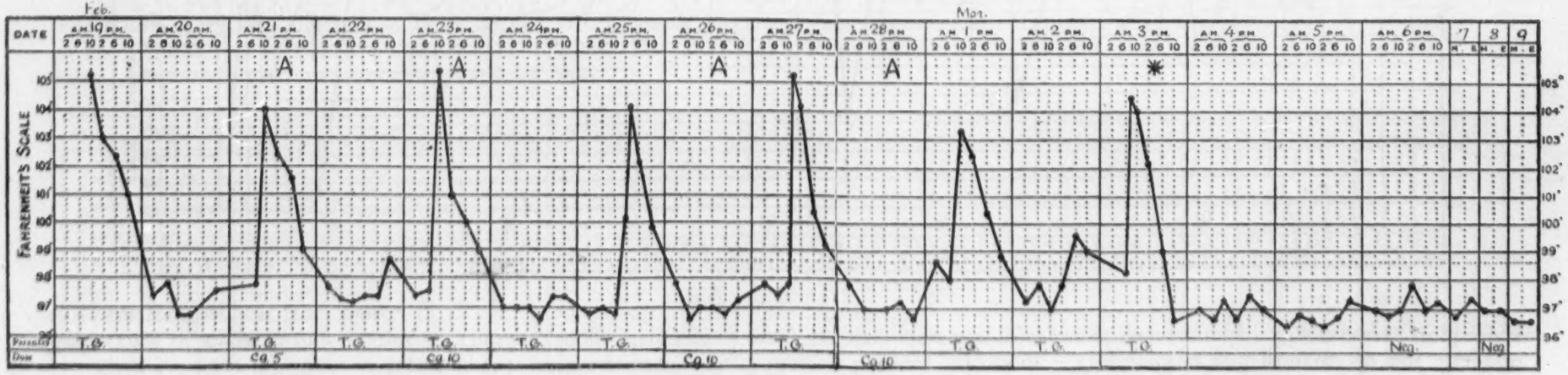
In the following temperature charts and tables:—

A = an injection of tartar emetic.
cg. = centigrammes of tartar emetic.
T = simple tertian trophozoites or schizonts.
G = simple tertian gametes.
t = malignant tertian trophozoites or schizonts.
cr. = malignant tertian gametes.
Neg. = No parasites found.
• = quinine orally given daily.
† = cessation of quinine treatment.

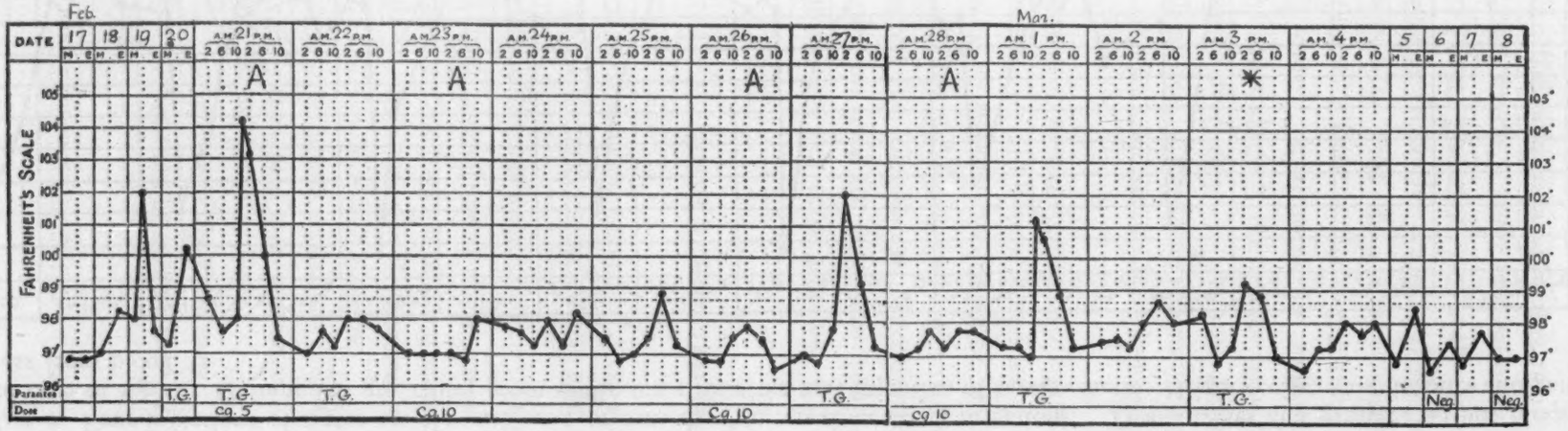
CASE 1. Two injections of tartar emetic, with one day's interval between injections. Trophozoites and gametes do not disappear. Continuance of rigors. Quinine (20 grs. daily) given orally nine days after first injection of tartar emetic. Rigors cease; trophozoites and gametes disappear from blood.



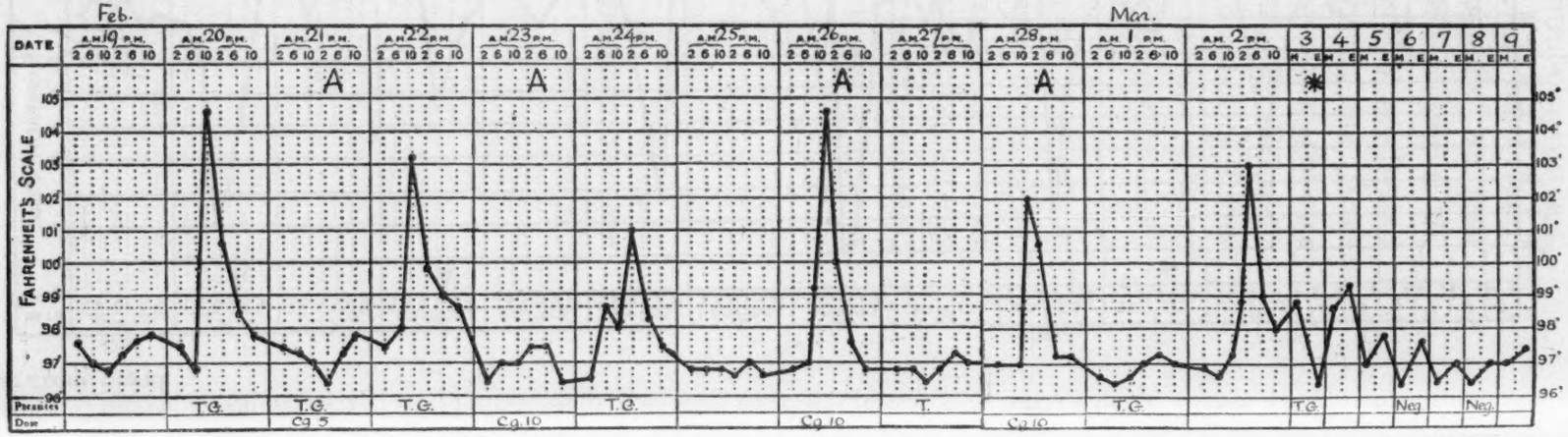
CASE 2. Four injections of tartar emetic with one or two days' interval between injections. Trophozoites and gametes do not disappear. Continuance of rigors. Quinine (20 grs. daily) given orally ten days after first injection of tartar emetic. Rigors cease; trophozoites and gametes disappear from blood.



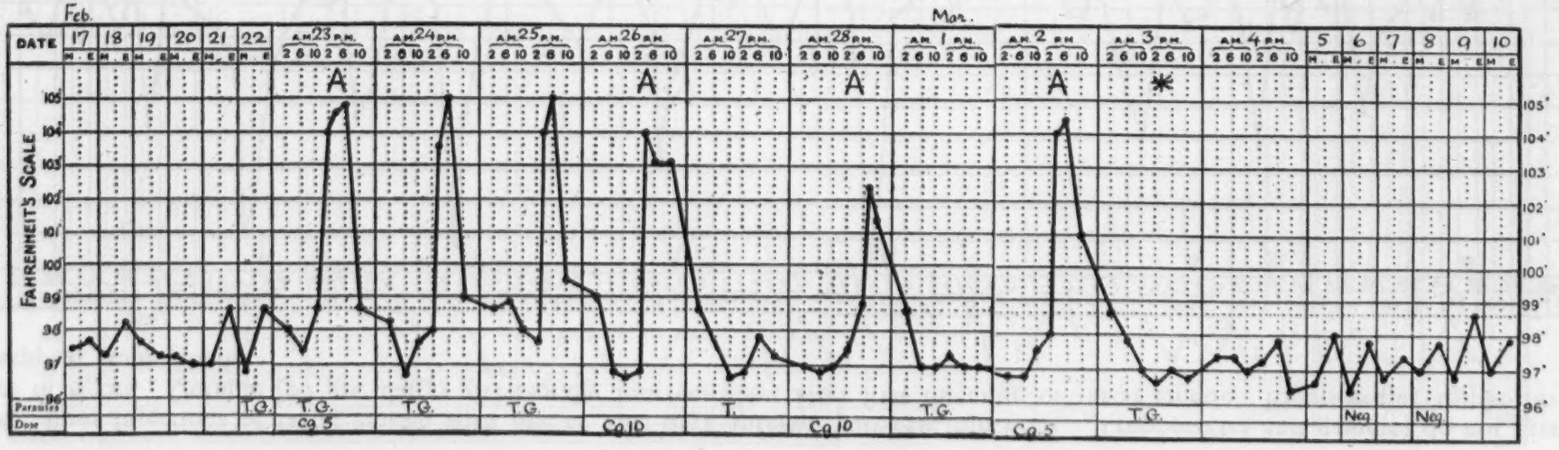
CASE 3. Four injections of tartar emetic, with one or two days' interval between injections. Trophozoites and gametes do not disappear. Recurrence of rigors during treatment. Quinine (20 grs. daily) given orally ten days after first injection of tartar emetic. Rigors cease; trophozoites and gametes disappear from blood.



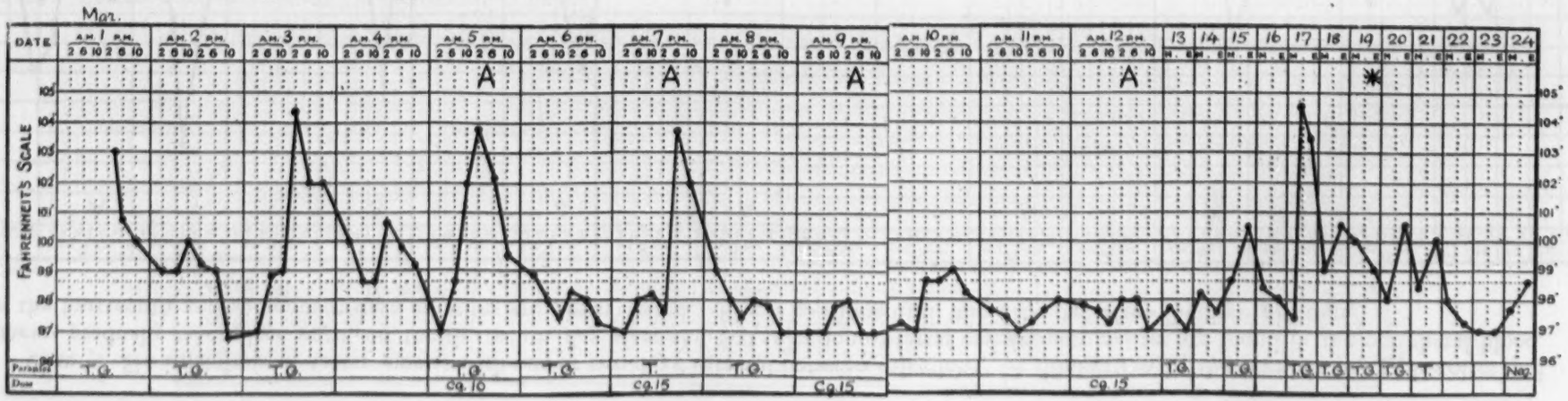
CASE 4. Four injections of tartar emetic with one or two days' interval between injections. Trophozoites and gametes do not disappear. Continuance of rigors. Quinine (20 grs. daily) given orally ten days after first injection of tartar emetic. Rigors cease; trophozoites and gametes disappear from blood.



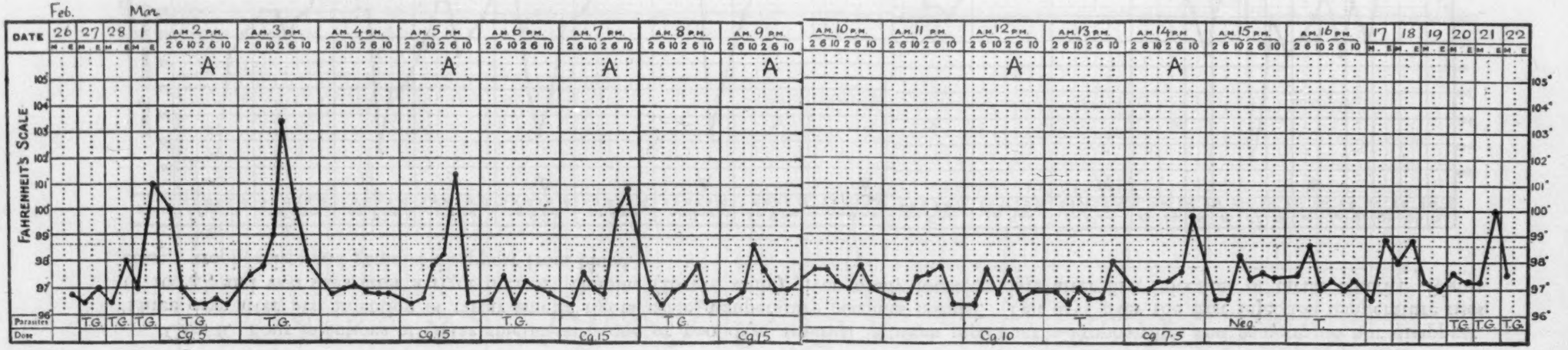
CASE 5. Four injections of tartar emetic with one or two days' interval between injections. Trophozoites and gametes do not disappear. Continuance of rigors. Quinine (20 grs. daily) given orally eight days after first injection of tartar emetic. Rigors cease; trophozoites and gametes disappear from blood.



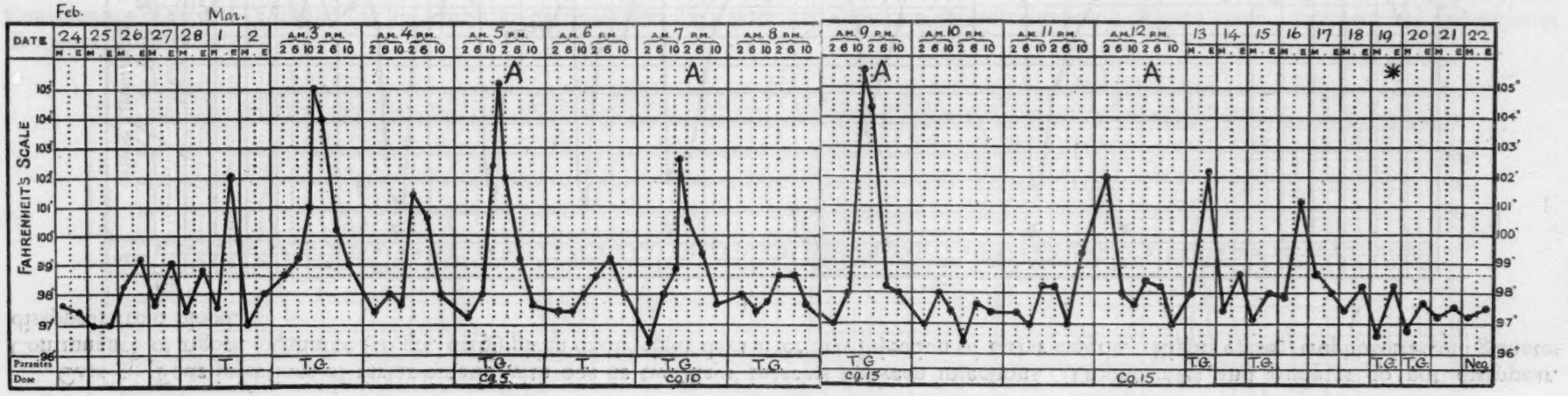
CASE 6. Four injections of tartar emetic with one or two days' interval between injections. Trophozoites and gametes do not disappear. Three days after first injection of tartar emetic rigors cease, temperature becomes normal. Ten days after the first injection temperature rises to 100·4°, and two days later a rigor occurs. Quinine (20 grs. daily) given orally fourteen days after first injection of tartar emetic. Rigors cease; trophozoites and gametes disappear from blood.



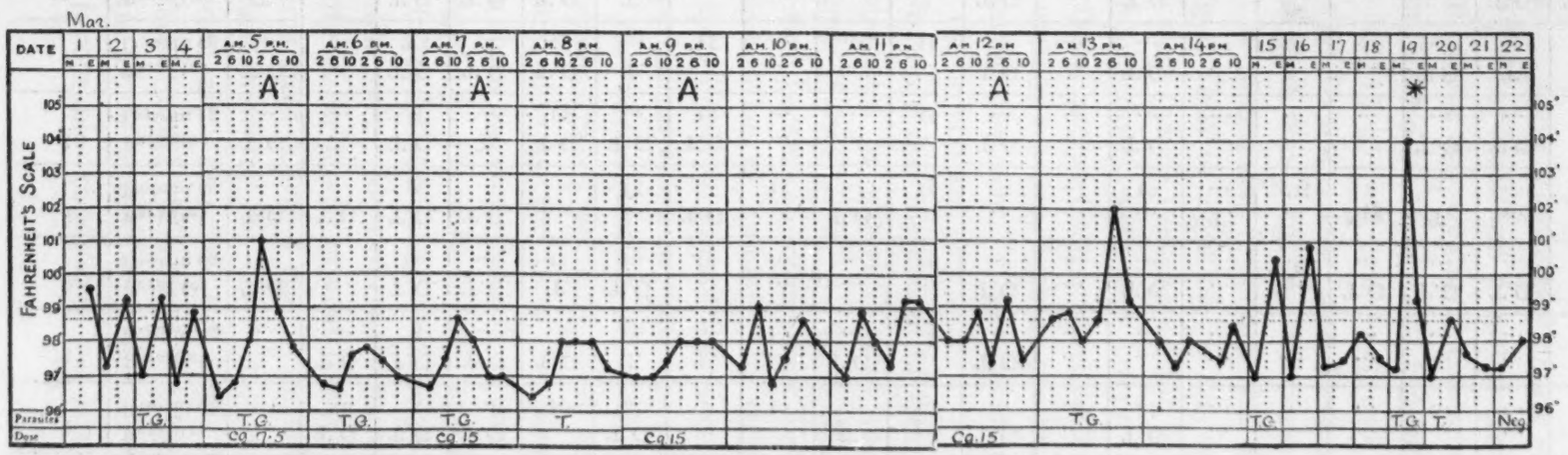
CASE 7. Six injections of tartar emetic with one or two days' interval between injections. A diminution in the number of trophozoites and gametes gradually takes place until, on the thirteenth day after the first injection of tartar emetic, no parasites are found in the peripheral blood. On the fourteenth and subsequent days parasites are again found. Rigors continue until the fifth day after the first injection.



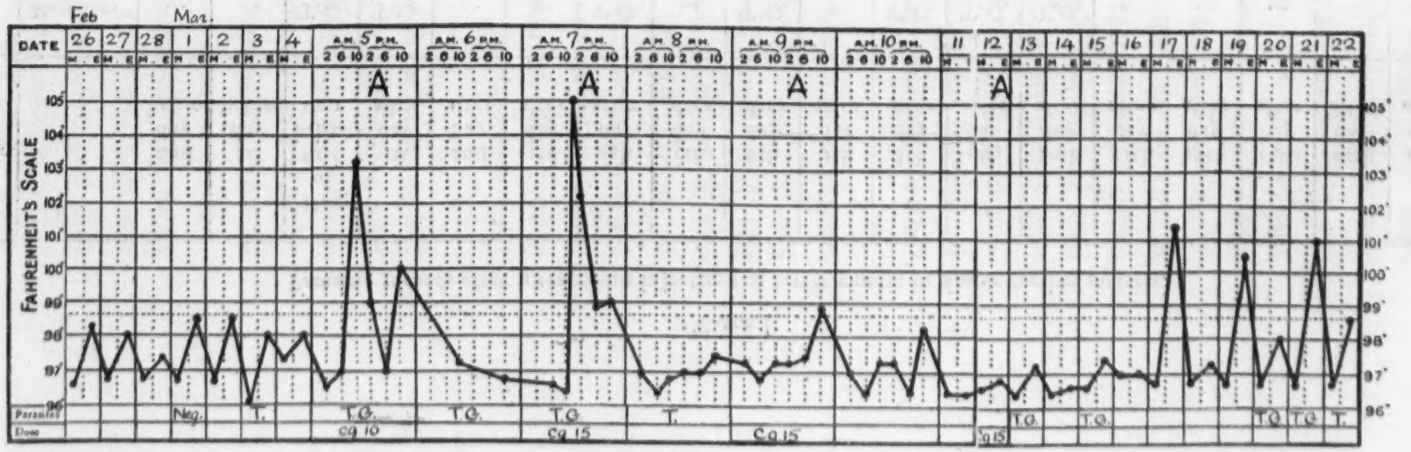
CASE 8. Four injections of tartar emetic with one or two days' interval between injections. Trophozoites and gametes do not disappear. Continuance of rigors. Quinine (20 grs. daily) given orally fourteen days after first injection of tartar emetic. Rigors cease; trophozoites and gametes disappear from blood.



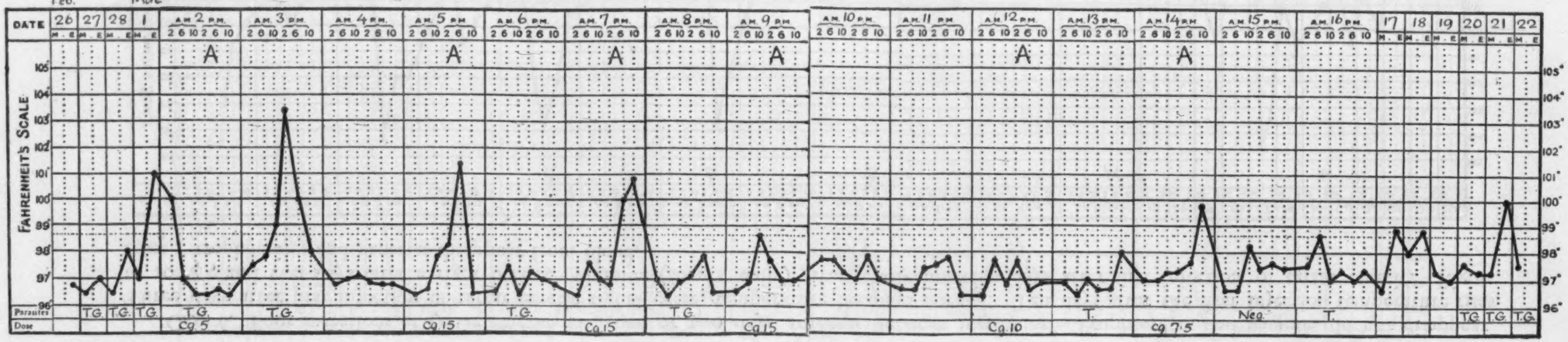
CASE 9. Four injections of tartar emetic with one or two days' interval between injections. Trophozoites and gametes do not disappear. The patient has no rigors either during the four days before treatment nor while treatment continues. On the fourteenth day after the first injection a rigor occurs, and quinine (20 grs. daily) is given orally. There is no further rise of temperature; trophozoites and gametes disappear from blood.



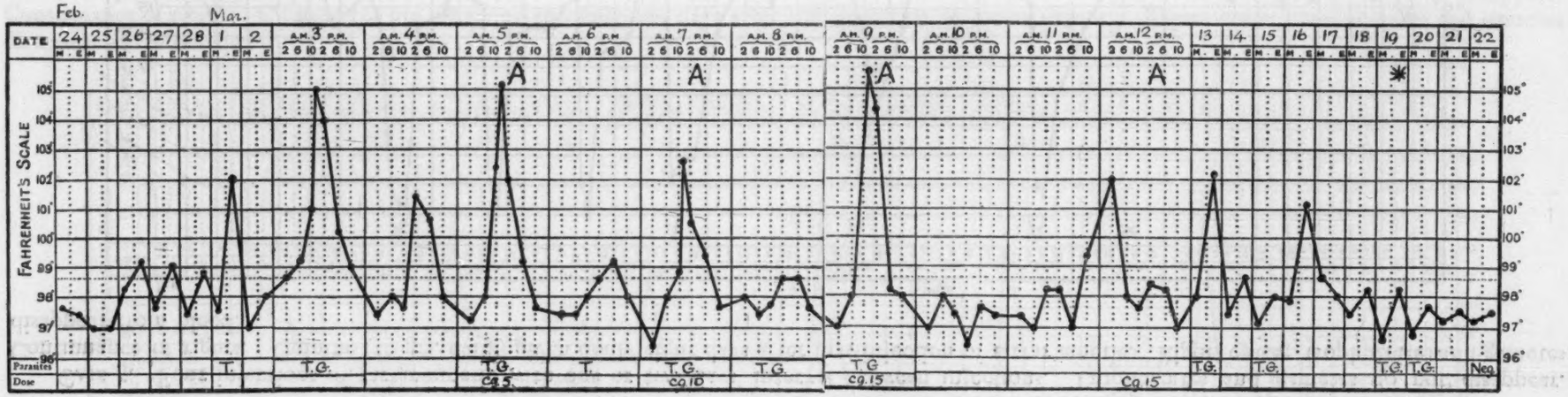
CASE 10. Four injections of tartar emetic with one or two days' interval between injections. Trophozoites and gametes do not disappear. Rigors cease three days after the first injection, and the temperature remains normal until the twelfth day after the first injection, when tertian paroxysms recur.



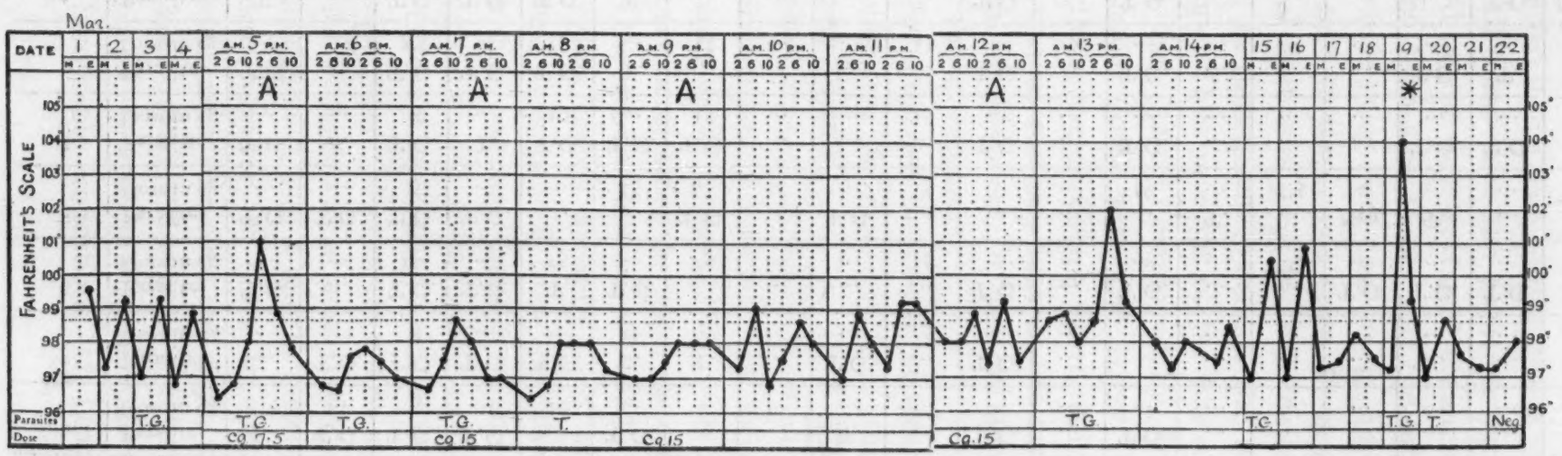
CASE 7. Six injections of tartar emetic with one or two days' interval between injections. A diminution in the number of trophozoites and gametes gradually takes place until, on the thirteenth day after the first injection of tartar emetic, no parasites are found in the peripheral blood. On the fourteenth and subsequent days parasites are again found. Rigors continue until the fifth day after the first injection.



CASE 8. Four injections of tartar emetic with one or two days' interval between injections. Trophozoites and gametes do not disappear. Continuance of rigors. Quinine (20 grs. daily) given orally fourteen days after first injection of tartar emetic. Rigors cease; trophozoites and gametes disappear from blood.



CASE 9. Four injections of tartar emetic with one or two days' interval between injections. Trophozoites and gametes do not disappear. The patient has no rigors either during the four days before treatment nor while treatment continues. On the fourteenth day after the first injection a rigor occurs, and quinine (20 grs. daily) is given orally. There is no further rise of temperature; trophozoites and gametes disappear from blood.



CASE 10. Four injections of tartar emetic with one or two days' interval between injections. Trophozoites and gametes do not disappear. Rigors cease three days after the first injection, and the temperature remains normal until the twelfth day after the first injection, when tertian paroxysms recur.

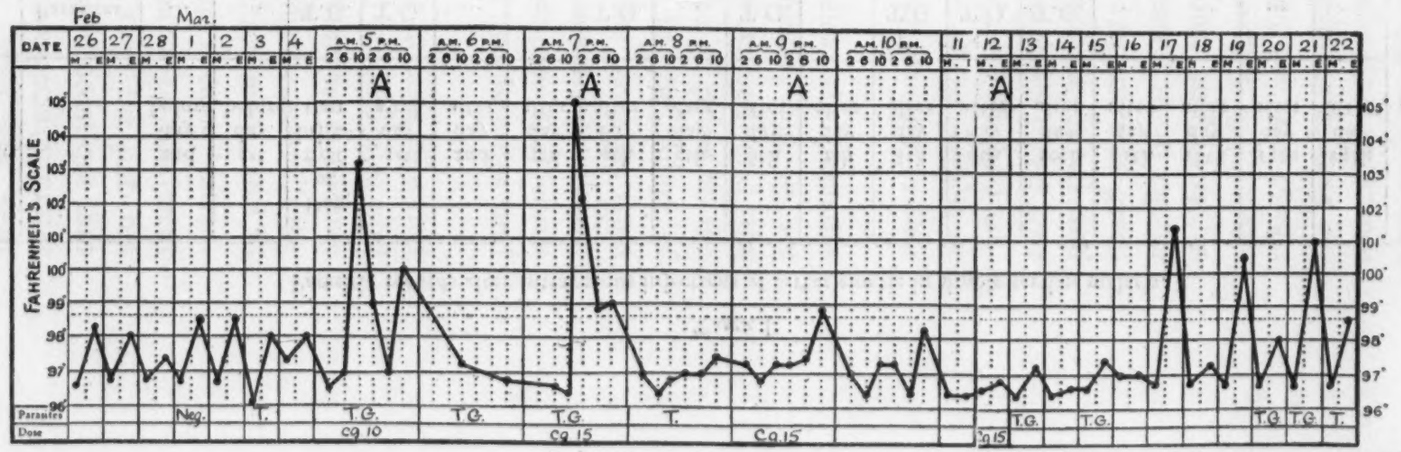


TABLE I

Parasitic records after intravenous injections of tartar emetic in simple tertian malaria.

Number of Case		2nd day before	1st day before	Day of first injection	1st day after	2nd day after	3rd day after	4th day after	5th day after	6th day after	7th day after	8th day after	9th day after	10th day after	11th day after	12th day after	13th day after	14th day after	15th day after	16th day after
1	Parasites	...	...	T.G.	T.G.	...	...	T.G.	...	T.G.	...	T.G.	T.G.	T.G.	...	...	...	...	...	...
	Dose in cg.	...	...	5	...	10	...	...	...	...	...	...	...	...	...	...	...	...	...	...
2	Parasites	T.G.	...	T.G.	T.G.	T.G.	T.G.	T.G.	...	T.G.	...	T.G.	T.G.	T.G.	...	...	...	...	...	...
	Dose in cg.	...	...	5	...	10	...	...	10	...	10	...	...	...	...	...	...	...	...	...
3	Parasites	...	T.G.	T.G.	T.G.	...	...	...	...	T.G.	...	T.G.	...	T.G.	...	...	...	...	...	...
	Dose in cg.	...	...	5	...	10	...	...	10	...	10	...	...	...	...	...	...	...	...	...
4	Parasites	...	T.G.	T.G.	T.G.	...	T.G.	...	...	T.	...	T.G.	...	T.G.	...	...	...	...	...	...
	Dose in cg.	...	...	5	...	10	...	...	10	...	10	...	...	...	...	...	...	...	...	...
5	Parasites	...	T.G.	T.G.	T.G.	T.G.	...	T.	...	T.G.	...	T.G.	...	...	...	...	...	...	...	...
	Dose in cg.	...	...	5	...	...	10	...	10	...	5	...	...	...	...	...	...	...	...	...
6	Parasites	T.G.	...	T.G.	T.G.	T.	T.G.	...	...	...	...	T.G.	...	T.G.	...	...	T.G.	T.G.	T.G.	T.
	Dose in cg.	...	...	10	...	15	...	15	...	...	15	...	...	...	...	T.G.	...	...	...	...
7	Parasites	T.G.	T.G.	T.G.	T.G.	...	...	T.G.	...	T.G.	...	...	...	...	T.	...	Neg.	T.	...	...
	Dose in cg.	...	...	5	...	...	15	...	15	...	15	...	...	10	...	7·5	...	...	...	...
8	Parasites	T.G.	...	T.G.	T.	T.G.	T.G.	T.G.	...	...	...	T.G.	...	T.G.	...	...	...	T.G.	T.G.	...
	Dose in cg.	...	...	5	...	10	...	15	...	...	15	...	...	...	...	...	...	...	...	...
9	Parasites	T.G.	...	T.G.	T.G.	T.G.	T.	...	...	...	...	T.G.	...	T.G.	...	...	...	T.G.	T.	...
	Dose in cg.	...	...	7·5	...	15	...	15	...	...	15	...	...	...	...	...	...	...	...	...
10	Parasites	T.	...	T.G.	T.G.	T.G.	T.	...	...	...	...	T.G.	...	T.G.	...	...	...	...	T.G.	T.G.
	Dose in cg.	...	...	10	...	15	...	15	...	...	15	...	...	...	...	...	...	...	...	...

The more important points observed in Cases 1 to 10 are recorded in the temperature charts and in Table 1.

All these cases had parasites (trophozoites and gametes) in their blood on the day treatment commenced. The individual injections varied from 5 to 15 cg. and the total amounts from 15 to 67·5 cg. (one case 15 cg., one case 30 cg., three cases 35 cg., one case 45 cg., one case 52·5 cg., two cases 55 cg., and one case 67·5 cg.). The initial dose was 5 cg. in seven cases, 7·5 cg. in one, and 10 cg. in two; in subsequent injections the dose was increased to 10 cg. or 15 cg. Thus in eight of the ten cases a larger amount was given than that (32 cg.) employed by Rogers, and both the initial and maximum doses were also larger. All the cases were given four injections, except Case 1 which had two and Case 7 which had six. For various reasons, such as the serious condition of the patient or refusal of treatment, it was not found possible to proceed further with the injections.

Not only did the course of treatment fail to clear the parasites (trophozoites or gametes) from the cutaneous blood, but in six patients (Cases 1, 2, 3, 4, 5, 8) the rigors continued and the condition became so serious that we were compelled to resort to the administration of quinine. In striking contrast to the failure of antimony either to control the fever or eradicate the parasites from the peripheral blood in these six cases, was the action of quinine, after the administration of which, in every instance, the febrile paroxysms ceased abruptly, and parasites, both trophozoites and gametes, disappeared from the cutaneous blood.

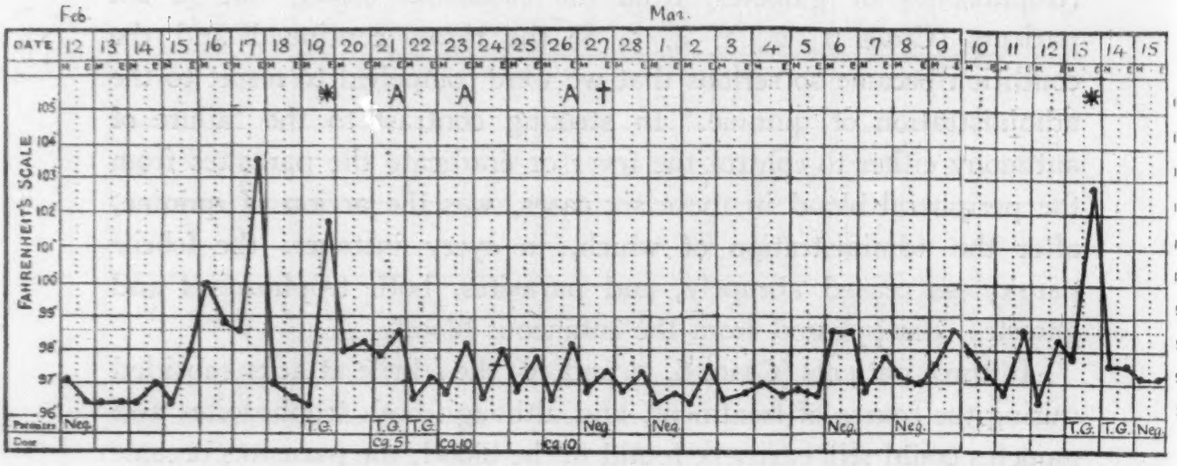
In three patients (Cases 6, 7, and 10) the febrile attacks subsided during the course of treatment, and although both trophozoites and gametes could still easily be found in the blood, the parasites became less numerous. Most of the charts indeed show some decrease in the severity of the febrile attacks during the course of treatment, and in two instances (Cases 5 and 8) quotidian paroxysms became tertian. It must, however, be borne in mind that the course of treatment extended as a rule over ten days, during which time the patients were kept in bed. Moreover, it is hardly necessary to point out that malaria febrile attacks may subside spontaneously, especially under the favourable conditions of hospital régime. This has been

repeatedly observed, e.g., by Laveran (1893), Mannaberg (1894), and others.

Apart from all this, one fact is clearly brought out, namely that tartar emetic in the doses given failed to cause the disappearance from the peripheral blood of either trophozoites or gametes in any of the ten cases.

Rogers, in the commentary at the end of his paper, suggests 'that quinine should be used to check the malarial paroxysms, while tartar emetic should subsequently be given intravenously, in the hope that it may prove of value in destroying the extracorpuscular stages of the malaria parasites, and so prevent relapses.' This combined treatment was tried in the following case:—

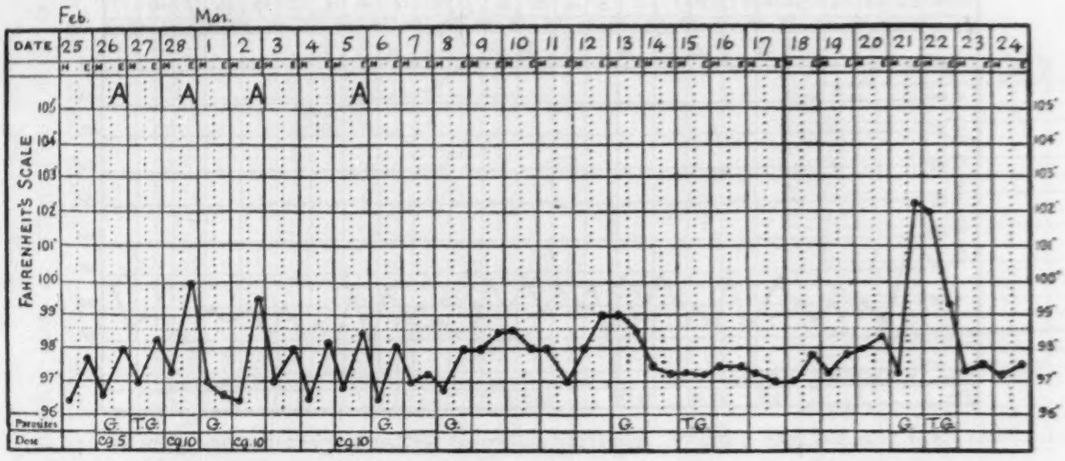
CASE 11. Quinine (20 grs. daily) given orally from 19th-27th February. Three injections of tartar emetic on 21st, 23rd and 26th February. Under this combined treatment the fever subsides and parasites (trophozoites and gametes) disappear from the peripheral blood, but on 13th March patient has a rigor and parasites (trophozoites and gametes) are again found.



In this case the paroxysms were first checked by quinine, and during its continuance three injections of tartar emetic were administered, but in spite of this combined treatment, the patient relapsed with simple tertian malaria in fourteen days after cessation of treatment. Tartar emetic, therefore, did not prevent the relapse which usually occurs in simple tertian malaria after a short course of quinine.

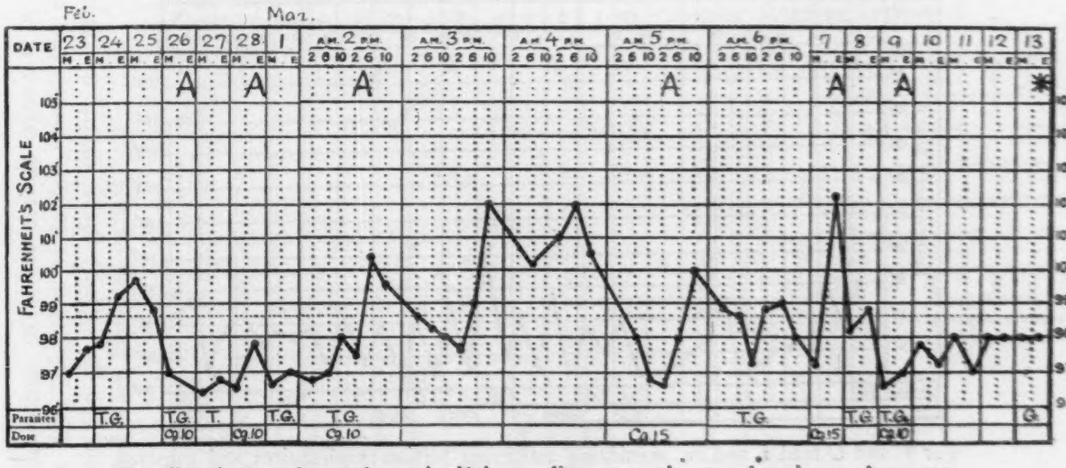
II. MALIGNANT TERTIAN MALARIA (Cases 12 to 21)

CASE 12. Four injections of tartar emetic with one or two days' interval between injections. Gametes do not disappear. Treatment commenced during an apyrexial period.



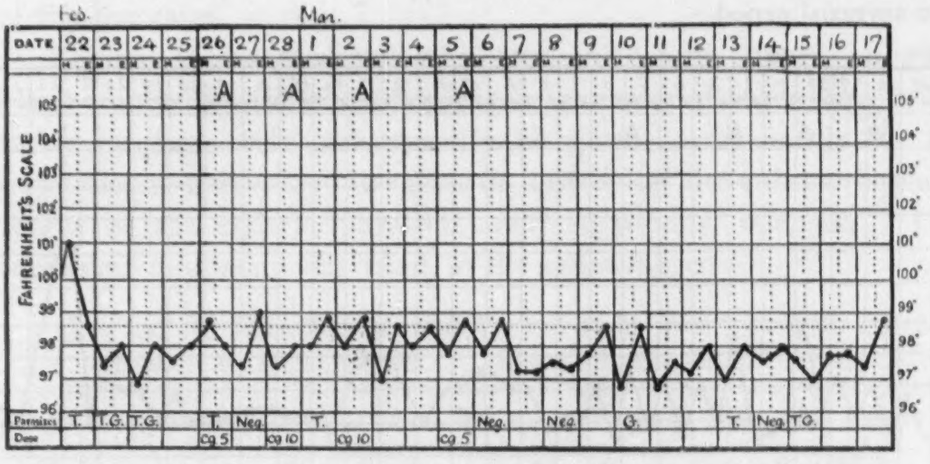
For *T* and *G* read *t.* and *cr.*, signifying malignant tertian trophozoites and gametes.

CASE 13. Six injections of tartar emetic with one or two days' interval between injections. Trophozoites and gametes do not disappear. At the commencement of treatment the temperature is normal and remains so until the fourth day after the first injection, when there occurs a series of rigors, which subside on the tenth day; gametes persist.



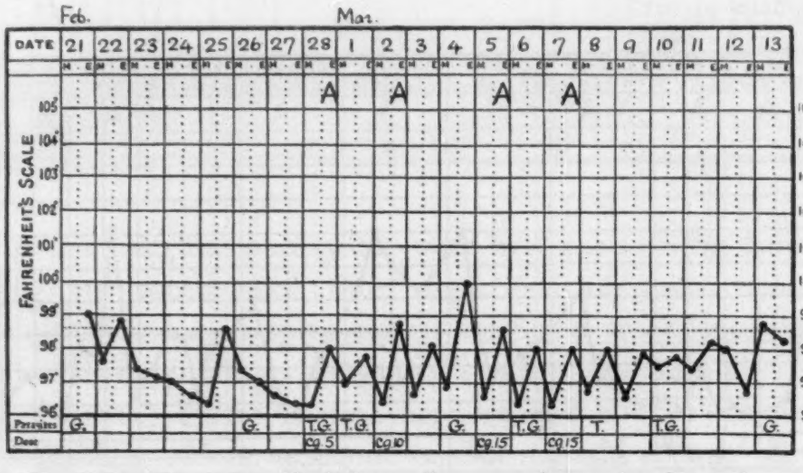
For *T* and *G* read *t.* and *cr.*, signifying malignant tertian trophozoites and gametes.

CASE 14. Four injections of tartar emetic with one or two days' interval between injections. Trophozoites only are found in the blood on the first day of treatment. Gametes are present two days before treatment commences and are again found on the fifth day after the last injection. Treatment administered during an apyrexial period.



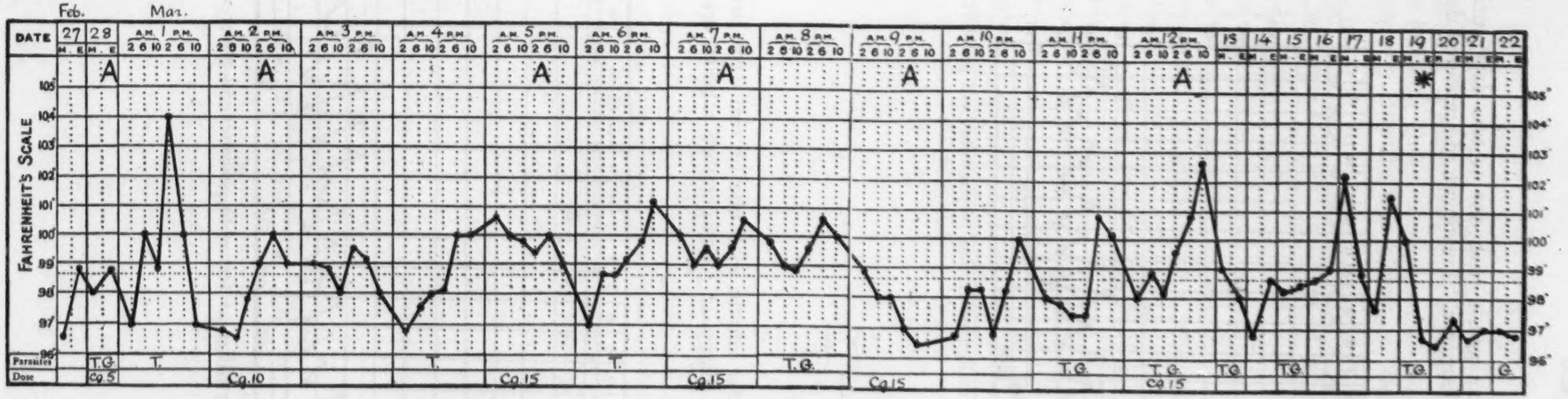
For *T* and *G* read *t.* and *cr.*, signifying malignant tertian trophozoites and gametes.

CASE 15. Four injections of tartar emetic with one or two days' interval between injections. Trophozoites and gametes do not disappear. Treatment commenced during an apyrexial period.



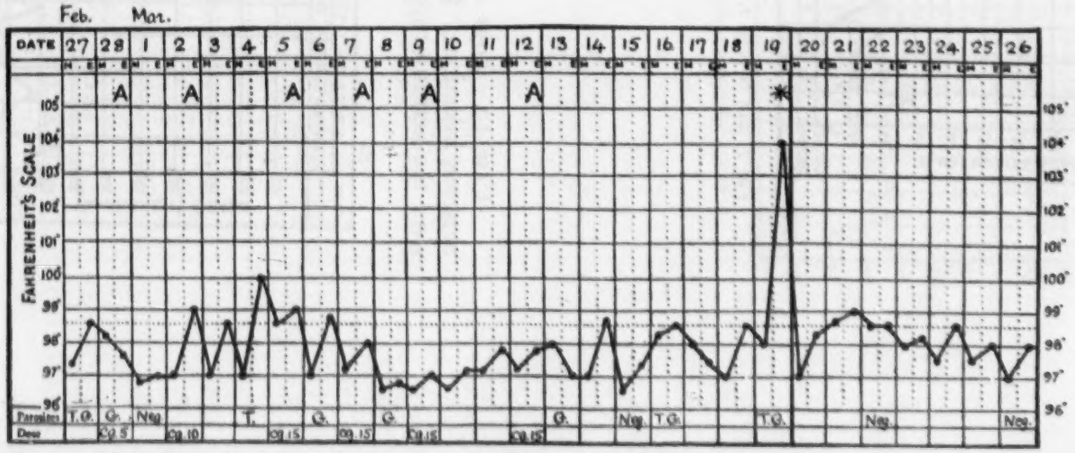
For *T* and *G* read *t.* and *cr.*, signifying malignant tertian trophozoites and gametes.

Case 16. Six injections of tartar emetic with one or two days' interval between injections. Trophozoites and gametes do not disappear. The day following the first injection of tartar emetic the patient has a rigor, and has further rises of temperature daily throughout the course of treatment. Quinine (20 grs. daily) given orally nineteen days after first injection of tartar emetic. Rigors cease; trophozoites disappear from the peripheral blood, but gametes persist.



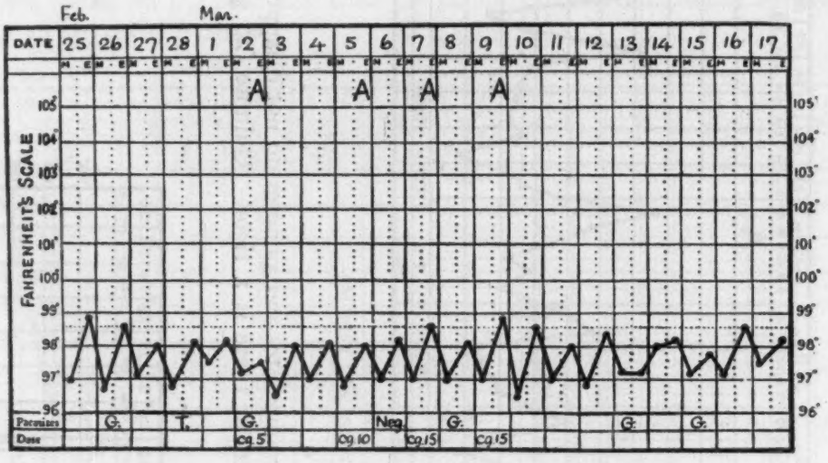
For *T* and *G* read *t.* and *cr.*, signifying malignant tertian trophozoites and gametes.

Case 17. Six injections of tartar emetic with one or two days' interval between injections. Trophozoites and gametes do not disappear. Treatment administered during an apyrexial period. Nineteen days after the first injection of tartar emetic there is a rigor. Quinine (20 grs. daily) given orally. Temperature subsides; trophozoites and gametes disappear.



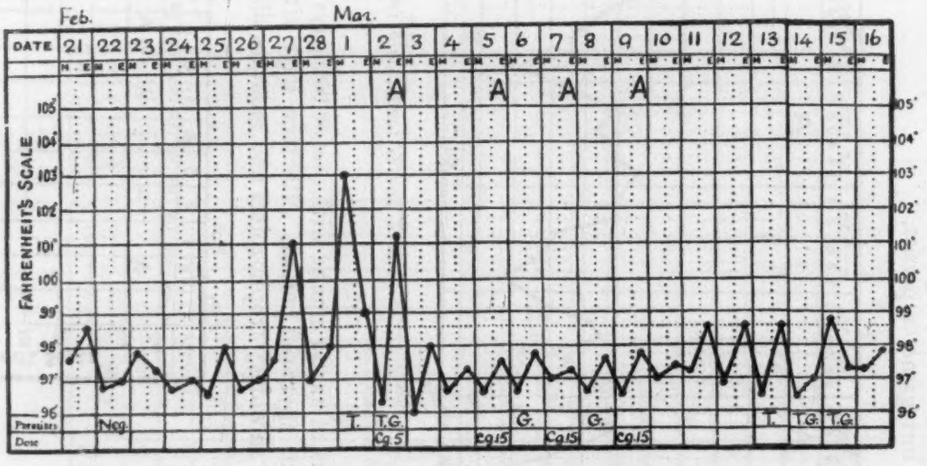
For *T* and *G* read *t.* and *cr.*, signifying malignant tertian trophozoites and gametes.

CASE 18. Four injections of tartar emetic with one or two days' interval between injections. Gametes do not disappear. Treatment administered during an apyrexial period.



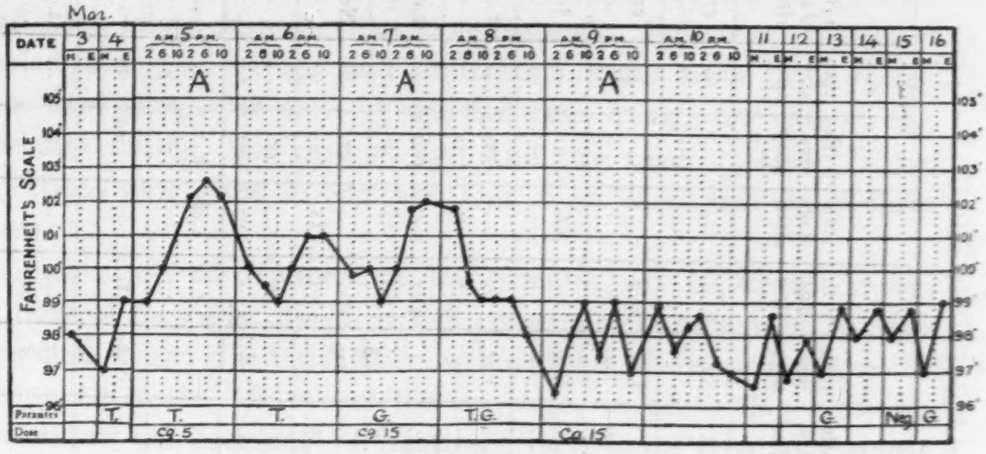
For *T* and *G* read *t.* and *cr.*, signifying malignant tertian trophozoites and gametes.

CASE 19. Four injections of tartar emetic with one or two days' interval between injections. Gametes do not disappear. Temperature subsides one day after the first injection.



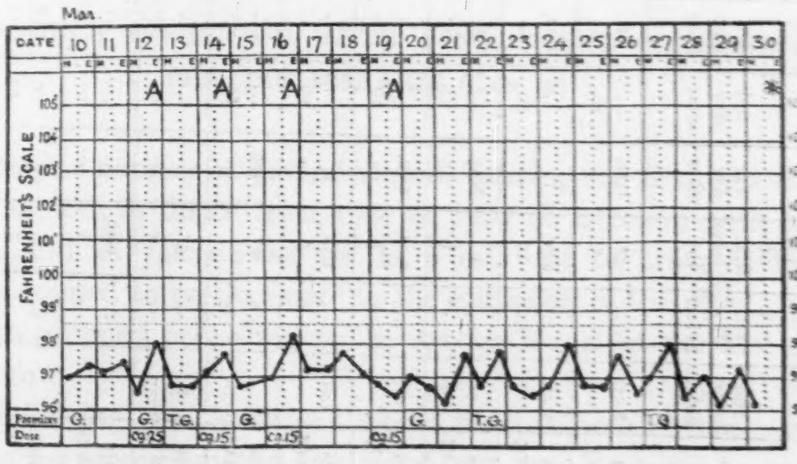
For *T* and *G* read *t.* and *cr.*, signifying malignant tertian trophozoites and gametes.

CASE 20. Three injections of tartar emetic with one day's interval between injections. Patient has a rigor on the day of the first injection and also on the two following days; temperature then falls to normal. Gametes appear on the second day after the first injection of tartar emetic and persist.



For *T* and *G* read *t.* and *cr.*, signifying malignant tertian trophozoites and gametes.

CASE 21. Four injections of tartar emetic with one or two days' interval between injections. Trophozoites and gametes do not disappear from blood. Treatment administered during an apyrexial period.



For *T* and *G* read *t.* and *cr.*, signifying malignant tertian trophozoites and gametes.

TABLE 2.

Parasitic records after intravenous injections of tartar emetic in malignant tertian malaria.

Number of Case		2nd day before	1st day before	Day of first injection	1st day after	2nd day after	3rd day after	4th day after	5th day after	6th day after	7th day after	8th day after	9th day after	10th day after	11th day after	12th day after	13th day after	14th day after	15th day after	16th day after	17th day after
12	Parasites	t.cr.	...	cr.	t.cr.	...	cr.	...	...	...	...	cr.	...	cr.	...	...	...	...	cr.	...	t.cr.
	Dose in cg.	...	...	5	...	10	...	10	...	...	10	...	...	...	...	...	...	...	...	...	...
13	Parasites	t.cr.	...	t.cr.	t.	...	t.cr.	t.cr.	...	...	...	t.cr.	...	t.cr.	t.cr.	...	...	...	cr.	...	cr.
	Dose in cg.	...	...	10	...	10	...	10	...	...	15	...	15	...	10	...	...	...	...	...	...
14	Parasites	t.cr.	...	t.	Neg.	...	t.	...	...	...	...	Neg.	...	Neg.	...	cr.	...	...	t.	Neg.	t.cr.
	Dose in cg	...	...	5	...	10	...	10	...	...	5	...	...	...	...	...	...	...	...	...	...
15	Parasites	cr.	...	t.cr.	t.cr.	...	...	cr.	...	t.cr.	...	t.	...	t.cr.	...	...	cr.	...	t.cr.	...	...
	Dose in cg.	...	...	5	...	10	...	...	15	...	15	...	...	...	...	...	...	...	...	...	...
16	Parasites	t.cr.	...	t.cr	t.	...	...	t.	...	t.	...	t.cr.	...	...	t.cr.	t.cr.	t.cr.	...	t.cr.	...	...
	Dose in cg.	...	...	5	...	10	...	...	15	...	15	...	15	...	...	15	...	...	...	...	...
17	Parasites	...	t.cr.	cr.	Neg.	...	...	t.	...	cr.	...	cr.	...	...	...	...	cr.	...	Neg.	t.cr.	...
	Dose in cg.	...	...	5	...	10	...	...	15	...	15	...	15	...	...	15	...	...	...	...	...
18	Parasites	t.	...	cr.	...	...	...	Neg.	...	cr.	...	...	...	...	cr.	...	cr.	...	...	...	...
	Dose in cg.	...	...	5	...	...	10	...	15	...	15	...	...	...	...	...	...	...	...	...	...
19	Parasites	...	t.	t.cr.	...	...	...	cr.	...	cr.	...	...	...	...	t.	t.cr.	t.cr.	...	...	...	...
	Dose in cg.	...	...	5	...	...	15	...	15	...	15	...	...	...	...	...	...	...	...	...	...
20	Parasites	...	t.	t.	t.	cr.	t.cr.	...	...	...	...	cr.	...	Neg.	cr.	...	...	...	...	t.	t.cr.
	Dose in cg.	...	...	5	...	15	...	15	...	...	...	...	...	...	...	...	...	...	...	...	...
21	Parasites	cr.	...	cr.	t.cr.	...	cr.	...	...	...	...	cr.	...	t.cr.	...	...	...	...	t.cr.	...	...
	Dose in cg.	...	...	7.5	...	15	...	15	...	...	15	...	...	...	...	...	...	...	...	...	...

The more important points observed in Cases 12 to 21 are recorded in the temperature charts and in Table 2.

All the cases had parasites in their peripheral blood on the day treatment commenced (four had gametes alone, four had gametes and trophozoites, two had trophozoites alone; in the two last gametes had been found at a previous examination). Individual injections varied from 5 to 15 cg., and the total amounts from 30 to 75 cg. (one case 30 cg., two cases 35 cg., two cases 45 cg., one case 50 cg., one case 60 cg., one case 70 cg., and two cases 75 cg.). Three injections were given in one case, four in six cases and six in three cases.

The effects of treatment on the gametes may be stated briefly. In none of the eight cases in which gametes were present on the day treatment began did the injections cause them to disappear from the peripheral blood. In one of the two cases in which they were not present on the first day of treatment they made their appearance two days later, while in the other they appeared on the fifth day after treatment ceased.

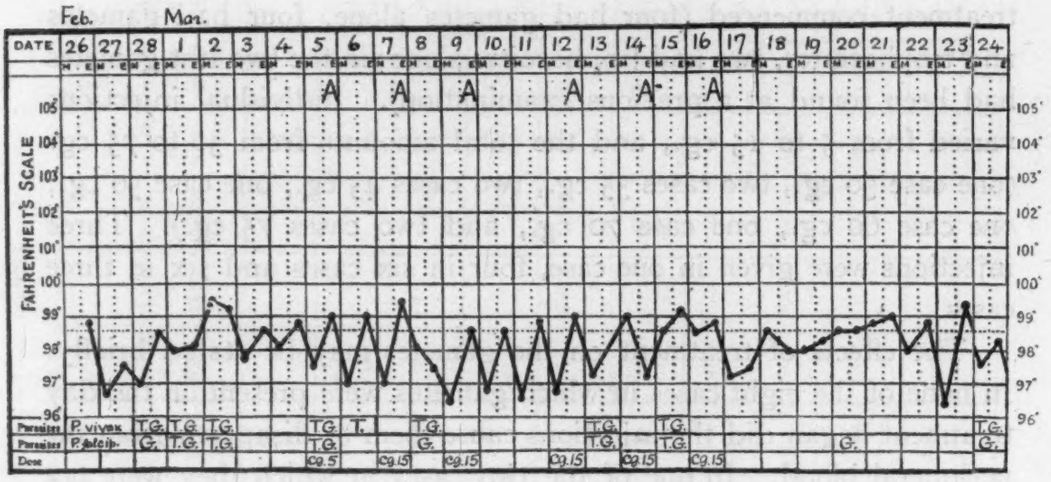
Low and Newham (1917) have also recorded the results of treating 'a case of heavy crescent infection' by this method; they found 'that the antimony had not the slightest effect on the crescents.'

III. DOUBLE INFECTION WITH SINGLE AND MALIGNANT TERTIAN MALARIA (Case 22)

Although a number of the individuals forming the first two series of cases showed evidence from time to time of infection with both *P. vivax* and *P. falciparum*, yet as a rule one or other of these infections seemed to predominate to the exclusion of the other; such cases were grouped according to the species found on the first day of treatment.

The following case is classed alone, because both *P. vivax* and *P. falciparum* were found in the blood not only previous to treatment, but also throughout the course of treatment.

CASE 22. Six injections of tartar emetic with one or two days' interval between injections. Trophozoites and gametes (*P. vivax* and *P. falciparum*) do not disappear. Treatment administered during an apyrexial period.



The only remark necessary concerning this case is that six injections of tartar emetic failed to cause the trophozoites and gametes of either species to disappear from the peripheral blood.

Reference may here be made to some of the symptoms produced by intravenous injection of tartar emetic. In this series of twenty-two cases coughing was frequently observed and vomiting in eight cases, and collapse occurred twice in the same patient. This last complication arose in a case of simple tertian infection (Case 5), who received injections during or immediately after a rigor on two occasions. So serious was the condition of this patient immediately following the injections that this treatment was discontinued.

CONCLUSIONS

1. Intravenous injections of tartar emetic do not cause the disappearance from the peripheral blood of any stage of the malaria parasites, whether *P. vivax* or *P. falciparum*.

2. These injections do not control either the rigors or the fever of acute malaria.

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STUDIES IN THE TREATMENT OF MALARIA

II. INTRAMUSCULAR INJECTIONS OF QUININE BIHYDROCHLORIDE IN SIMPLE TERTIAN MALARIA

BY

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Diverse views have been expressed in the past as to the value of intramuscular injections of quinine in the treatment of malaria. These views were based on clinical experience and experimental evidence. MacGilchrist (1911) states that 'Intramuscular injections are attended by the same drawbacks as subcutaneous proper; . . . *This mode of quinine administration should, therefore, be abandoned.*'

Sir Ronald Ross (1914), in reference to the value of intramuscular injections of quinine in malaria, wrote as follows: 'I really do not know why this form of medication is continued in malaria. . . . It appears to me that the only cases in which intramuscular injection is called for are those in which intestinal absorption may be checked by very marked intestinal affections, or where patients are not to be persuaded to take the drug by the

mouth at all. For severe cases, to judge by the experimental evidence, the subcutaneous injections are much worse than useless, because the patient may really not be absorbing any of the drug just at the critical moment when it is necessary that he should absorb a large quantity; and I fancy that a considerable number of the fatal cases are due to this. Where the drug cannot be given by the mouth in very severe cases, the intravenous injection in extreme dilution seems to be much preferable to the intramuscular one. I am aware that opposite opinions have been cited, but do not think that their weight is sufficient to convince us.'

Again, later in the same year, Ross states: 'In spite of the experiments referred to, these injections still appear to be largely used, and the matter may be worth mentioning again, because, in my opinion, the injections are quite possibly responsible for many of the fatalities which occur, though seldom, in malaria. The clinician imagines that he has given a dose sufficient to reduce the parasites below danger point, whereas, as a matter of fact, his dose generally remains *in situ*, and if the case is a dangerous one the parasites are allowed to breed unchecked in the patient's blood.'

Still more recently (1917) he writes: 'in fact, it may almost be maintained that the large case mortality in malaria may be greatly due to the use of such intramuscular injections.'

A controversy as to the merits of intramuscular injection in malaria followed the publication of these letters. Those interested can find the necessary references in the *Tropical Diseases Bulletin*, 1914.

It seemed to us, after reading the views of those who took part in this polemic, that it was a matter of urgency to determine whether intramuscular injection is a valuable mode of administering quinine or one which may be 'much worse than useless.'

In this paper we are not concerned with the relative merits of the various modes of quinine administration. The objects we had in view were merely to obtain evidence whether the following treatment (1) controlled the febrile paroxysms; (2) cleared the parasites (trophozoites and gametes) out of the cutaneous blood.

The treatment was an intramuscular injection of quinine bihydrochloride (grns. 15 in 2 c.c. of water) on each of two consecutive days. The injections were made into the deltoids.

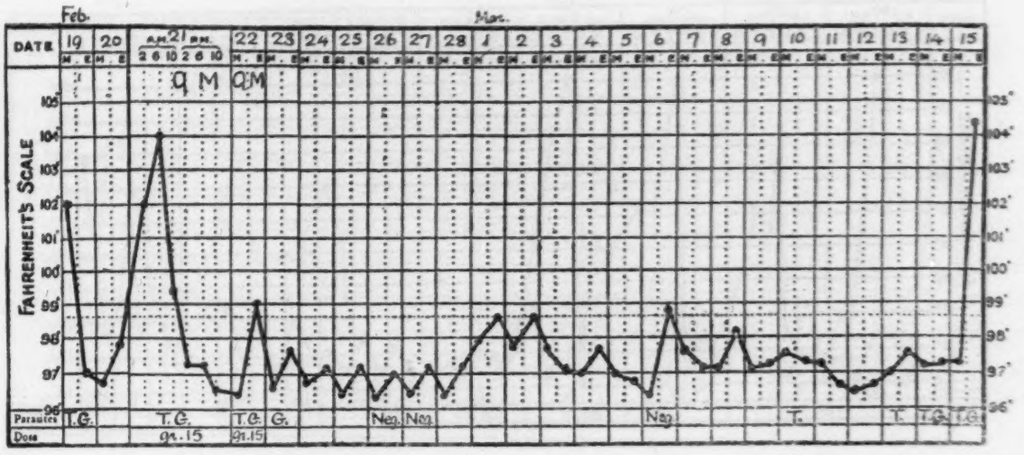
SIMPLE TERTIAN MALARIA (CASES 23—42)

All these cases were adult males infected in Macedonia at least six months previously, and all had had more or less quinine during this period. The observations made on the individual cases are recorded in the following charts, and the more important points are summarised in the tables (pp. 122-4).

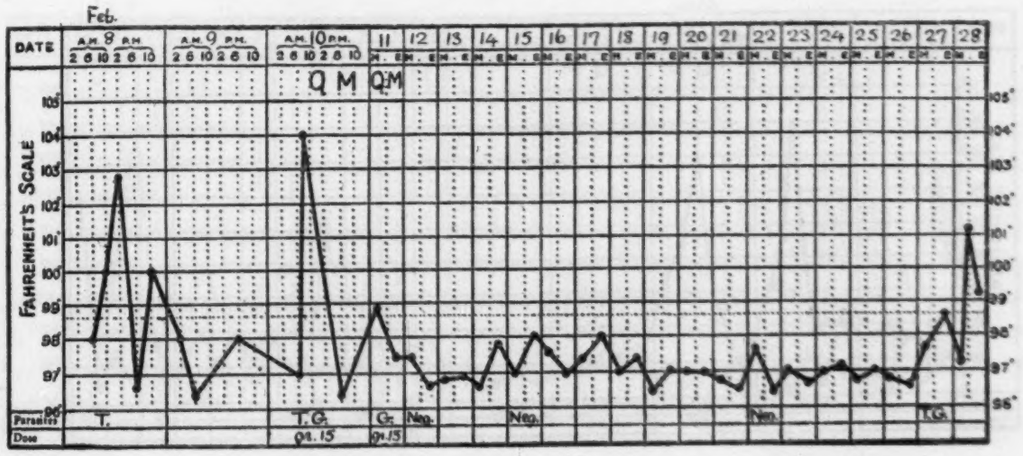
In the following temperature charts and tables:—

Q M = intramuscular injection of quinine.
gr. = grains of quinine bihydrochloride.
T = simple tertian trophozoites or schizonts.
G = simple tertian gametes.
Neg. = No parasites found.

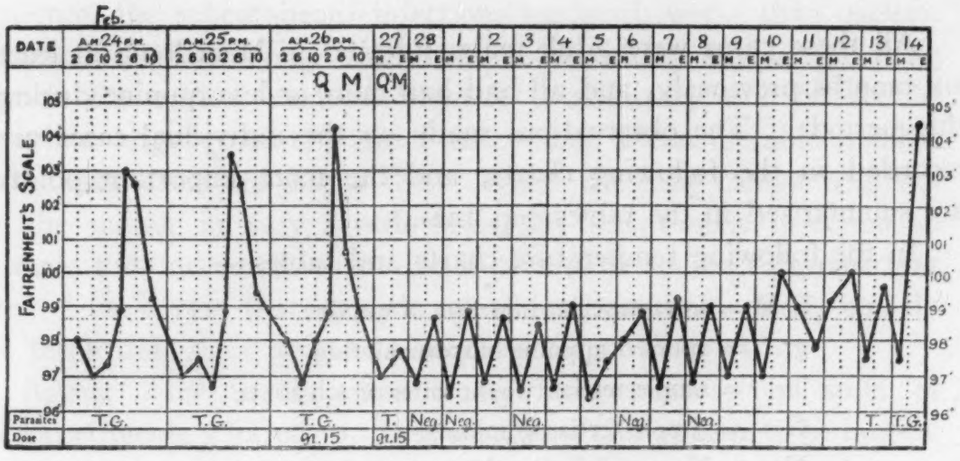
CASE 23.



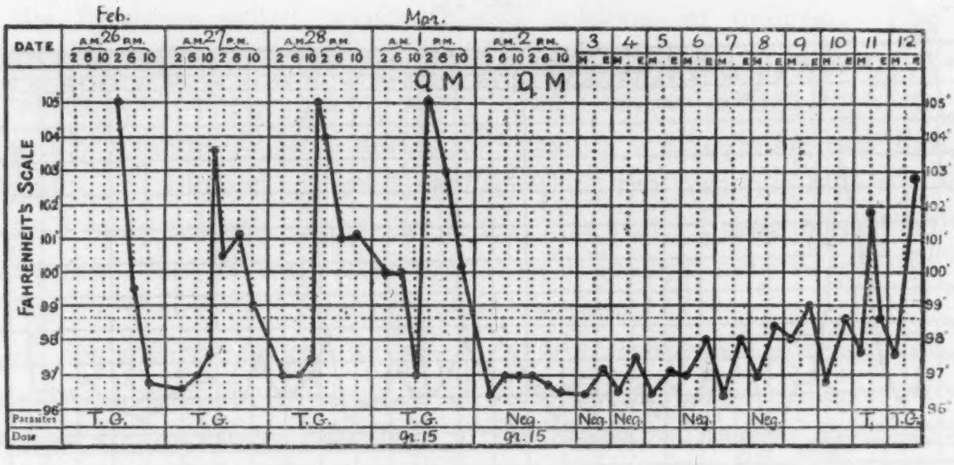
CASE 24.



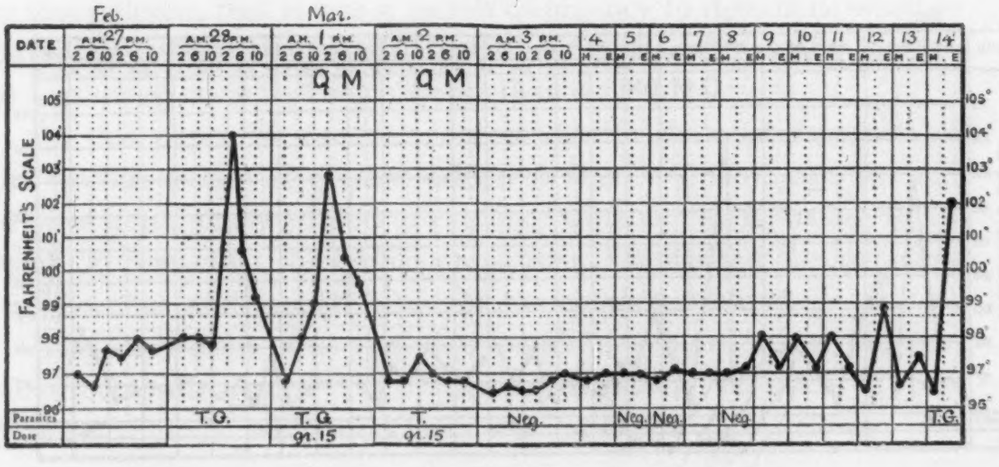
Case 25.



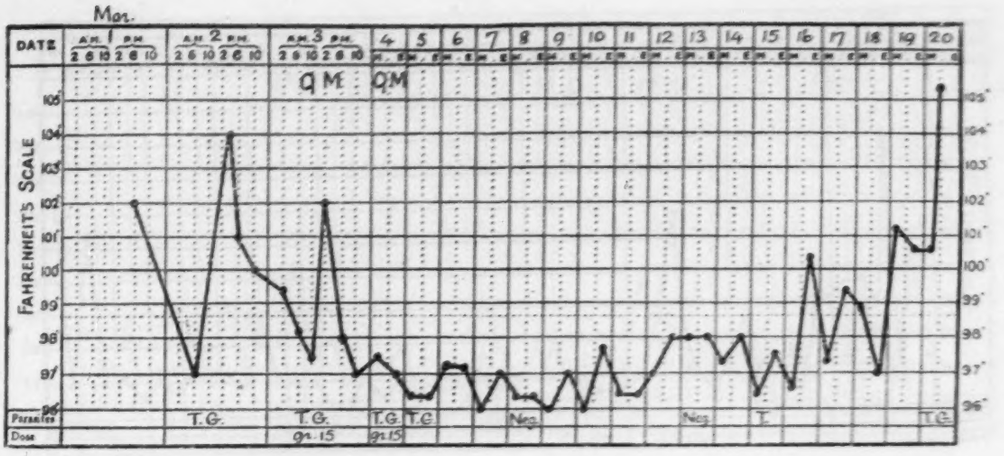
Case 26.



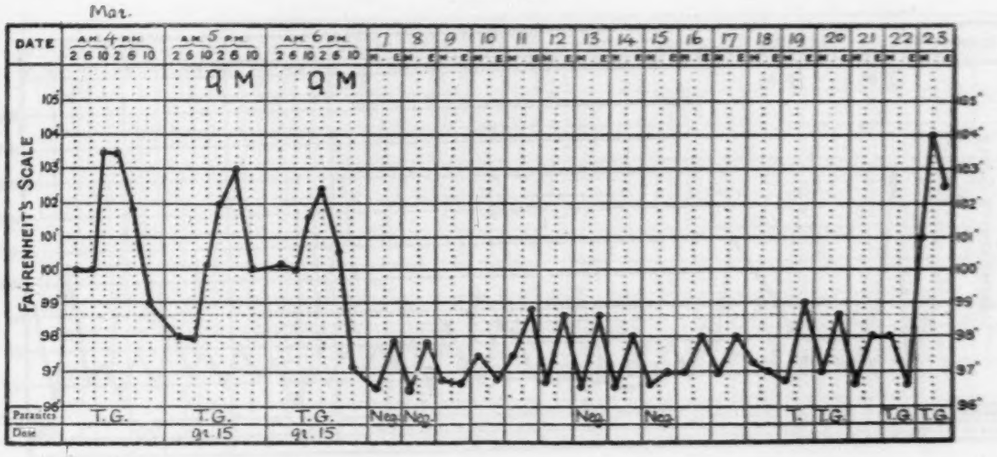
Case 27.



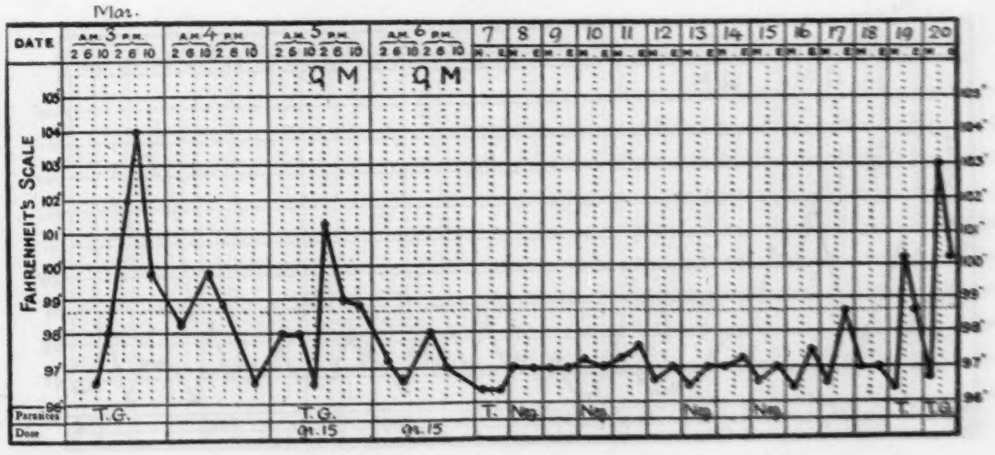
CASE 28.



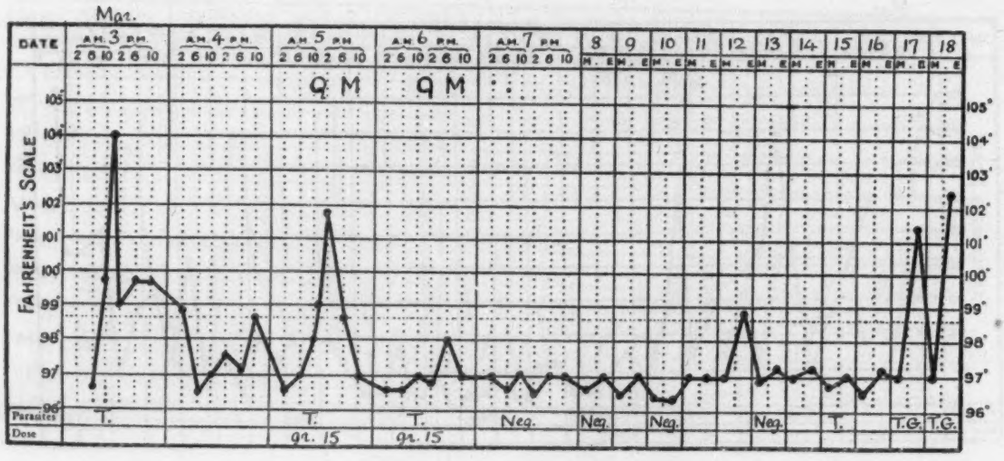
CASE 29.



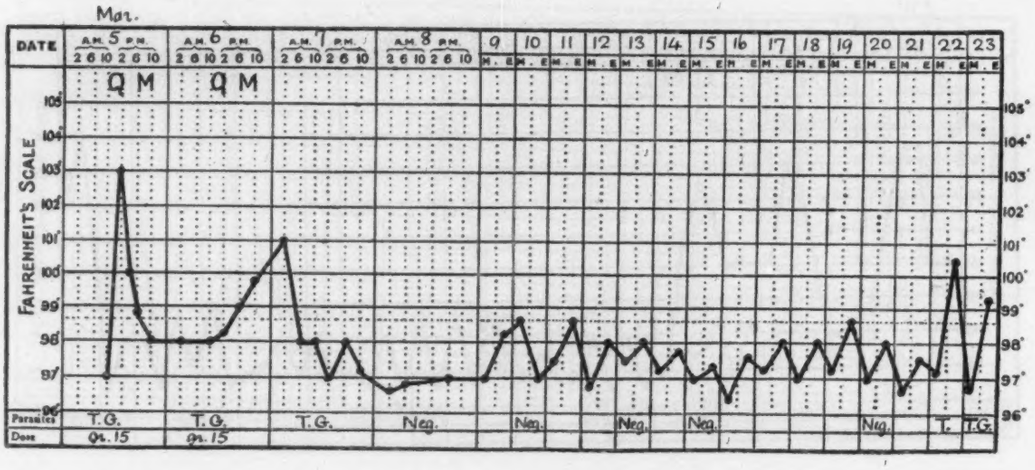
CASE 30.



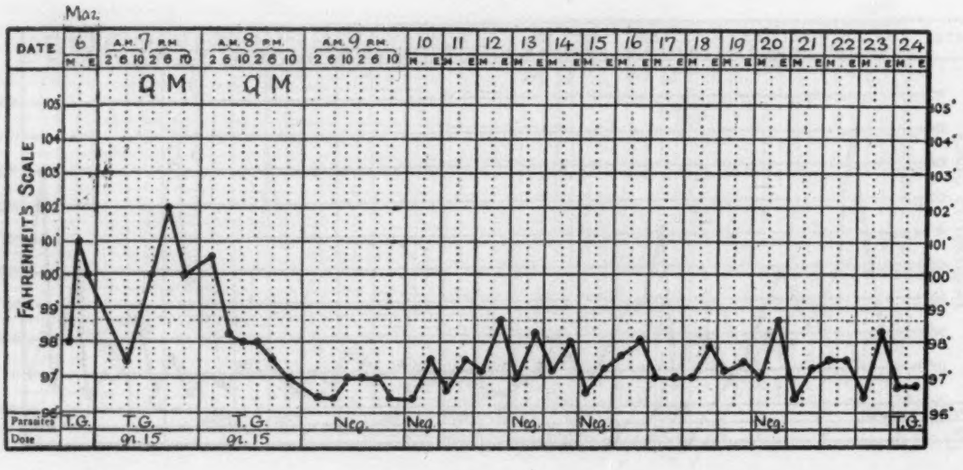
Case 31.



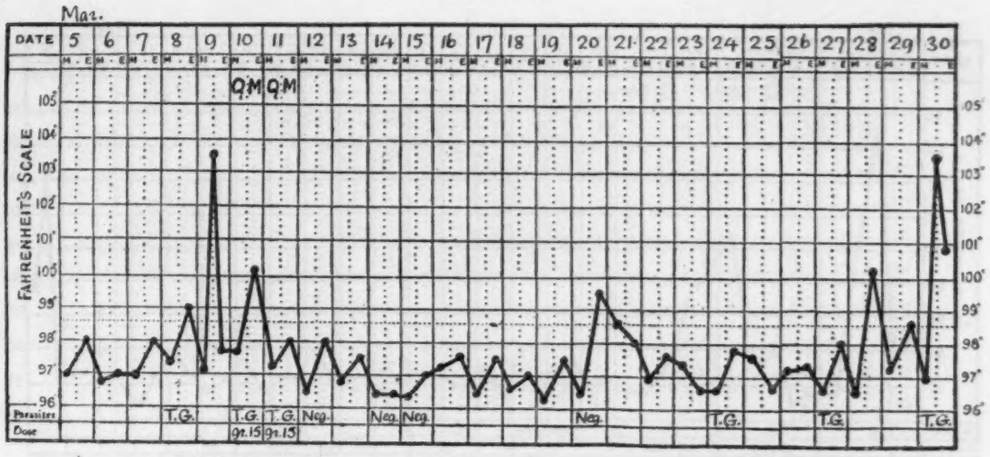
Case 32.



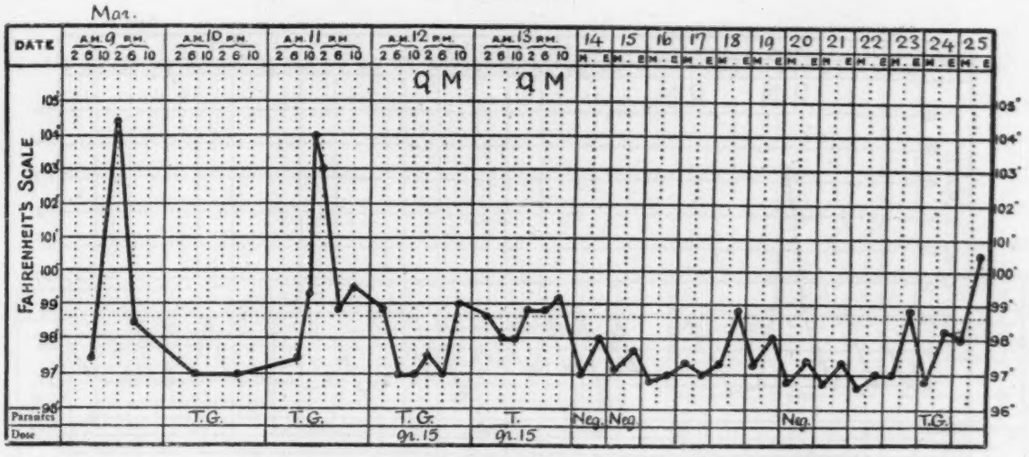
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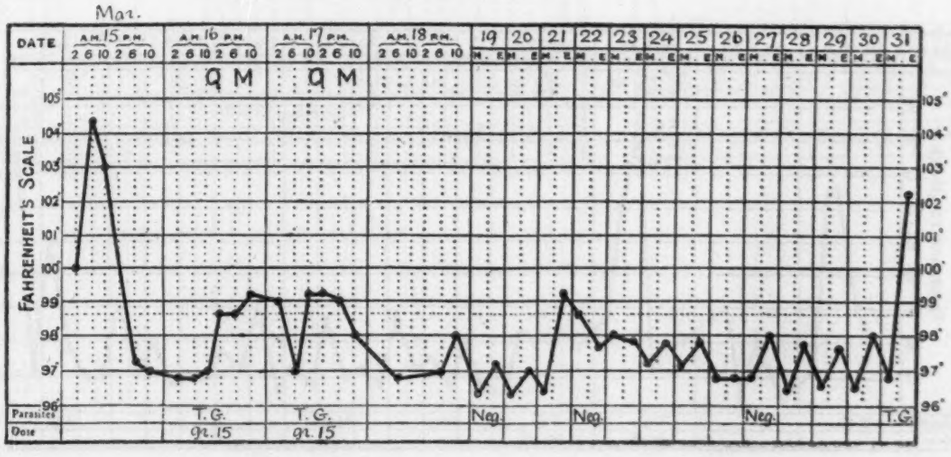
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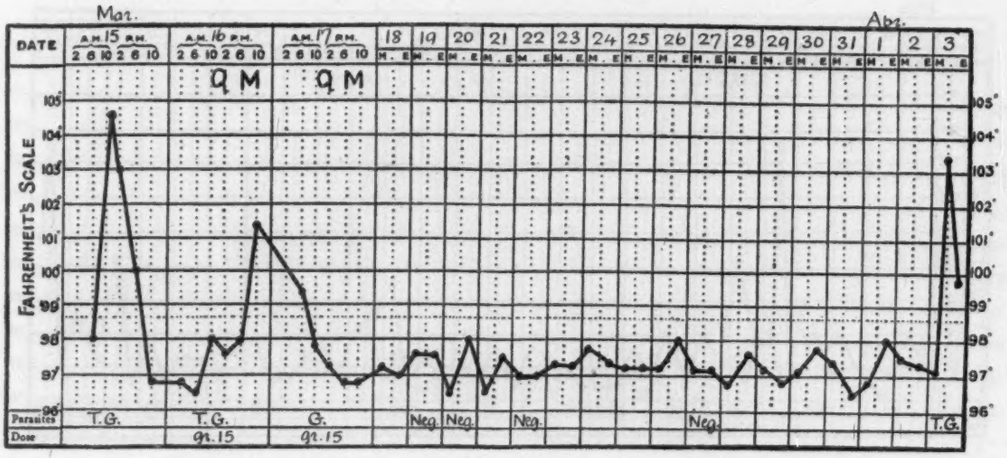
Case 35.



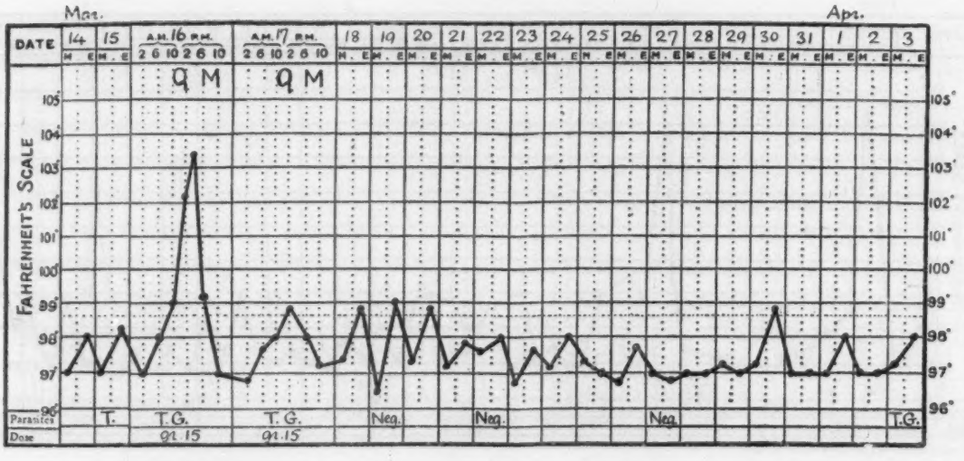
Case 36.



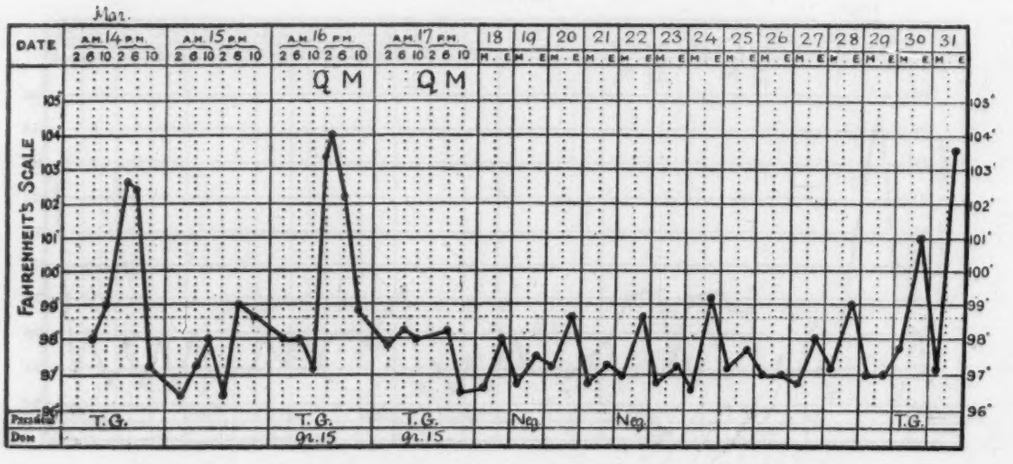
Case 37.



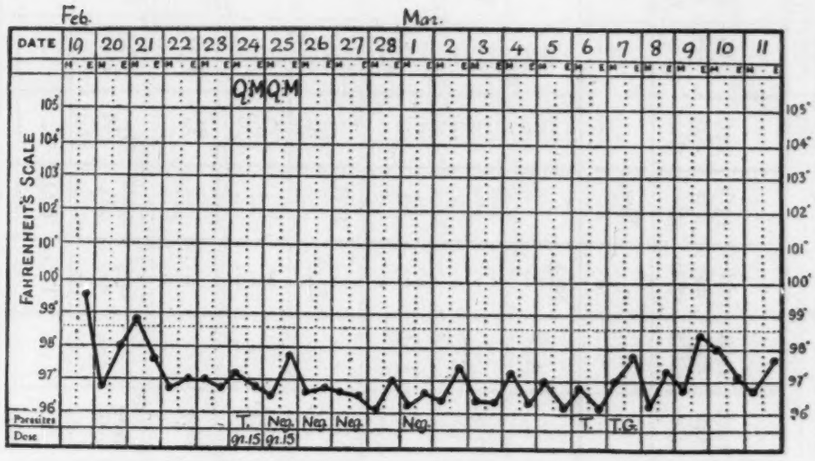
Case 38.



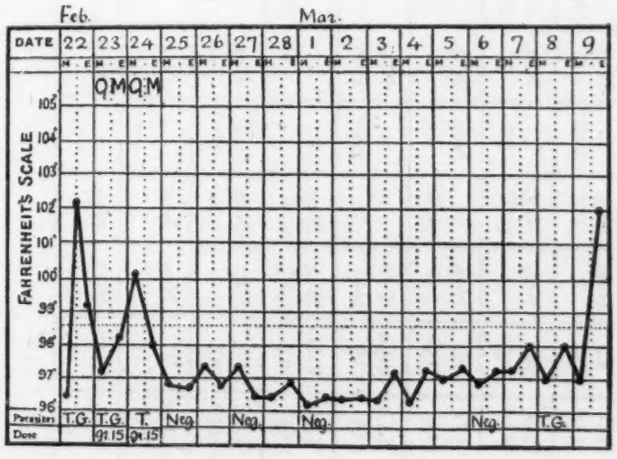
Case 39.



Case 40.



Case 41.



Case 42.

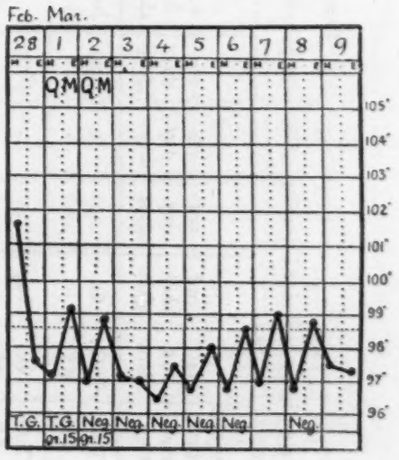


TABLE I.

Parasitic records after an intramuscular injection of quinine bihydrochloride on each of two consecutive days in simple tertian malaria.

Number of Case		2nd day before	1st day before	Day of first injection.	1st day after	2nd day after	3rd day after	4th day after	5th day after	6th day after	7th day after	8th day after	9th day after	10th day after	11th day after	12th day after	13th day after	14th day after	15th day after	16th day after	17th day after	18th day after
23	Parasites ...	T.G.	...	T.G.	T.G.	G.	...	...	Neg.	Neg.	...	...	...	...	...	...	Neg.	...	...	...	T.	...
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
24	Parasites ...	T.	...	T.G.	G.	Neg.	...	...	Neg.	...	...	...	...	...	...	Neg.	...	...	...	...	T.G.	...
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
25	Parasites ...	T.G.	T.G.	T.G.	T.	Neg.	Neg.	...	Neg.	...	...	Neg.	...	Neg.	...	...	...	...	T.	T.G.	...	...
	Dose in grains ...	...	...	15	15	...	...	...	...	...	—	...	...	...	...	...	...	...	...	...	...	...
26	Parasites ...	T.G.	T.G.	T.G.	Neg.	Neg.	Neg.	...	Neg.	...	Neg.	...	...	T.	T.G.	...	...	...	...	...	...	...
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
27	Parasites ...	...	T.G.	T.G.	T.	Neg.	...	Neg.	Neg.	...	Neg.	...	...	...	...	...	T.G.	...	...	...	...	...
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
28	Parasites ...	...	T.G.	T.G.	T.G.	T.G.	...	...	Neg.	...	...	...	...	Neg.	...	T.	...	...	...	...	T.G.	...
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
29	Parasites ...	...	T.G.	T.G.	T.G.	Neg.	Neg.	...	...	...	...	Neg.	...	Neg.	...	...	...	T.	T.G.	...	T.G.	TG.
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
30	Parasites ...	T.G.	...	T.G.	...	T.	Neg.	...	Neg.	...	...	Neg.	...	Neg.	...	...	...	T.	T.G.	...	...	...
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
31	Parasites ...	T.	...	T.	T.	Neg.	Neg.	...	Neg.	...	...	Neg.	...	T.	...	T.G.	T.G.	...	...	...	...	...
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
32	Parasites ...	...	...	T.G.	T.G.	T.G.	Neg.	...	Neg.	...	...	Neg.	...	Neg.	...	...	...	...	Neg.	...	T.	T.G.
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

TABLE I—*Continued.*

Number of Case		2nd day before	1st day before	Day of first injection.	1st day after	2nd day after	3rd day after	4th day after	5th day after	6th day after	7th day after	8th day after	9th day after	10th day after	11th day after	12th day after	13th day after	14th day after	15th day after	16th day after	17th day after	18th day after
33	Parasites ...	...	T.G.	T.G.	T.G.	Neg.	Neg.	...	...	Neg.	...	Neg.	...	...	...	...	Neg.	...	...	...	T.G.	...
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
34	Parasites ...	T.G.	...	T.G.	T.G.	Neg.	...	Neg.	Neg.	...	...	...	...	Neg.	...	...	...	T.G.	...	...	T.G.	...
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
35	Parasites ...	T.G.	T.G.	T.G.	T.	Neg.	Neg.	...	...	...	...	Neg.	...	...	...	T.G.	...	...	...	...	...	...
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
36	Parasites ...	...	...	T.G.	T.G.	...	Neg.	...	...	Neg.	...	...	...	...	Neg.	...	...	...	T.G.	...	...	...
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
37	Parasites ...	...	T.G.	T.G.	G.	...	Neg.	Neg.	...	Neg.	...	...	...	...	Neg.	...	...	...	...	...	...	T.G.
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
38	Parasites ...	...	T.	T.G.	T.G.	...	Neg.	...	...	Neg.	...	...	...	...	Neg.	...	...	...	...	...	...	T.G.
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
39	Parasites ...	T.G.	...	T.G.	T.G.	...	Neg.	...	...	Neg.	...	...	...	...	...	...	...	T.G.	...	...	...	...
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
40	Parasites ...	...	...	T.	Neg.	Neg.	Neg.	...	Neg.	...	...	...	...	T.	T.G.	...	...	...	...	...	...	...
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
41	Parasites ...	...	T.G.	T.G.	T.	Neg.	...	Neg.	...	Neg.	...	...	...	...	Neg.	...	T.G.	...	...	...	...	...
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
42	Parasites ...	...	T.G.	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	...	Neg.	...	...	...	...	...	Neg.	Neg.	...	...	...	Neg.
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

TABLE II.

Summary of results of intramuscular injections of quinine in simple tertian malaria.

Number of Case	Temperature fell to normal in — days after 1st injection	Parasites disappeared from cutaneous blood in — days after 1st injection	Parasitic relapse occurred in — days after 1st injection	Febrile relapse (above 100°F) occurred in — days after 1st injection	Remarks
23	1	3-5	14 — 17	22	...
24	1	2	13 — 17	18	...
25	1	2	11 — 15	16	...
26	1	1	8 — 10	10	...
27	1	2	8 — 13	13	...
28	1	3-5	11 — 12	13	...
29	2	2	11 — 14	18	...
30	1	3	11 — 14	14	...
31	1	2	9 — 10	12	...
32	2	3	16 — 17	17	...
33	1	2	14 — 17	...	Quinine 20 gr. orally on 17th day
34	1	2	11 — 14	18	...
35	1	2	9 — 12	13	...
36	1	2-3	12 — 15	15	...
37	1	2-3	12 — 18	18	...
38	1	2-3	12 — 18	...	Quinine 20 gr. orally on 18th day
39	1	2-3	7 — 14	14	...
40	Apyrexial period	1	6 — 10	...	Quinine 20 gr. orally on 12th day
41	2	2	12 — 13	14	...
42	1	1	...	...	No relapse within 2 months

In all these cases the treatment resulted in:—

1. Cessation of febrile paroxysms within two days of the first injection.

2. Disappearance of parasites (trophozoites and gametes) from the cutaneous blood.

Relapses. Nineteen of the twenty cases relapsed. Parasites reappeared on an average in 14 days (minimum period 10, maximum 18). These periods may have been slightly less, as blood examinations were not made every day. Febrile paroxysms recurred on an average in 15 days (minimum 10, maximum 22). The remaining case (Case 42) has not relapsed within the period of observation—two months.

Effects of the injections. Pain and tenderness following injection were so slight as to be practically negligible and had completely disappeared in two to three days, although we employed a concentrated solution (grns. 15 in 2 c.c., i.e. about 45 per cent.).

Controls. If control observations are desired, we refer the reader to the series of cases, treated by tartar emetic, recorded in our previous paper (p. 91).

CONCLUSION

An intramuscular injection of quinine bihydrochloride, grns. 15 in 2 c.c. of water, on each of two consecutive days causes the cessation of febrile paroxysms of simple tertian malaria and effects the disappearance of all stages of the parasite from the cutaneous blood. The action, however, is only temporary, a relapse occurring within two to three weeks.

It must be clearly understood that this refers to simple tertian malaria only. We hope to deal with malignant tertian malaria in a future paper.

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OBSERVATIONS ON THE PERIODICITY OF *MICROFILARIA NOCTURNA*

BY

WARRINGTON YORKE

AND

B. BLACKLOCK

(*Received for publication* 21 *May*, 1917)

PLATE III

The following is a brief history and clinical account of the case upon which the observations recorded in this paper were made:—

The patient, an Australian, 19 years of age, contracted the infection in Queensland. *Symptoms*—Pains in the back, accompanied by the voiding of urine of the appearance of 'milk tinged with blood' were noted for the first time in July, 1915. After a week or ten days the symptoms disappeared. In October, 1915, the patient sailed for Egypt where he remained until May, 1916. From May until the end of September he was in France. Whilst in Egypt and France urinary symptoms reappeared on four occasions, and lasted for periods varying from a fortnight to more than a month. Pain in the back was a prominent symptom at the commencement of the disease, and has continued intermittently, more especially at night, to the present time. Pain was experienced in the urethra and hypogastrium on micturition, especially when passing clots. Apart from these symptoms the patient has enjoyed fair health; his weight (130 lbs.) has remained unchanged.

On admission to the Hospital of the Liverpool School of Tropical Medicine the urine was milky and tinged with blood. A diagnosis of filariasis was made by centrifuging a small quantity of the urine and finding microfilariae in the deposit. Blood films made during the daytime were either negative or contained only an odd microfilaria, whereas in those prepared at night microfilariae were found in considerable numbers. Thick dehaemoglobinised films were stained with haematein, and the morphology of the parasite examined. The larvae were identified as those of *Filaria bancrofti*.

NOCTURNAL PERIODICITY OF THE LARVAE IN THE CUTANEOUS BLOOD

A series of observations was made with the object of studying the periodicity of the microfilariae in the cutaneous blood. The number of larvae per cubic centimetre of cutaneous blood was estimated every two hours for a period of twenty-four hours; this was done on two occasions, viz., 21st-22nd December, 1916, and 5th-6th January, 1917. Furthermore, the number of larvae per cubic centimetre of venous blood (median-basilic vein) was determined, one examination being made at midnight, 21st-22nd December, and a series of examinations—one every six hours—on 5th-6th January.

Technique. The number of larvae in 1 c.c. of the cutaneous blood was ascertained in the following manner :—The tip of a finger was punctured with a surgical needle and ·02 c.c. of blood drawn into a graduated pipette. The measured volume of blood was then discharged on to six slides in approximately equal drops which were covered with slips $\frac{7}{8}$ inch square. Experience showed that six was the smallest number of such preparations which should be made from ·02 c.c. of blood; if a smaller number were made the films were so thick that the microfilariae were apt to be obscured by red blood corpuscles. The total number of larvae in the coverslip preparations was then counted and the number per cubic centimetre calculated; a low power of the microscope (Zeiss obj. AA., oc. 4) was used for this purpose. In the case of the venous blood about 2 c.c. were withdrawn from the median basilic vein by means of a small syringe; the blood was then immediately discharged into a watch glass and ·02 c.c. taken up in the graduated pipette; the subsequent steps in the examination were as described above.

While this method answered admirably when the larvae were fairly numerous (500 or more per cubic centimetre of blood) it was not satisfactory when they were very scanty (less than 100), as the number contained in ·02 c.c. of blood was too small. For this reason the following technique was employed when only small numbers of larvae were present. Instead of ·02 c.c. of blood ·2 c.c. was drawn into a graduated pipette, mixed with an equal volume of 1 per cent. sodium citrate dissolved in normal salt solution, and centrifuged in a wide capillary tube. The supernatant fluid was then pipetted off and the capillary tube cut through well below the level of the upper limit of the red cell column. The contents of the upper portion of the cut capillary tube were blown out on slides, and the end of the tube washed out with a drop of normal saline which was also discharged upon a slide. The preparations were then covered with slips $\frac{7}{8}$ inch square, and the microfilariae enumerated as before.

The results, which are given in Tables 1 and 2, in Graph 1 and in Graph 2, Curve 1, show clearly the nocturnal periodicity of the larvae in the cutaneous blood. The maximum concentration observed was 12,850 larvae per cubic centimetre of cutaneous blood at midnight 21st-22nd December, and 12,100 at 1 a.m. 5th-6th January. Although during the daytime the number of larvae fell to a low level, on no occasion were they completely absent from the cutaneous blood, the minimum noted being 50 per cubic centimetre of blood.

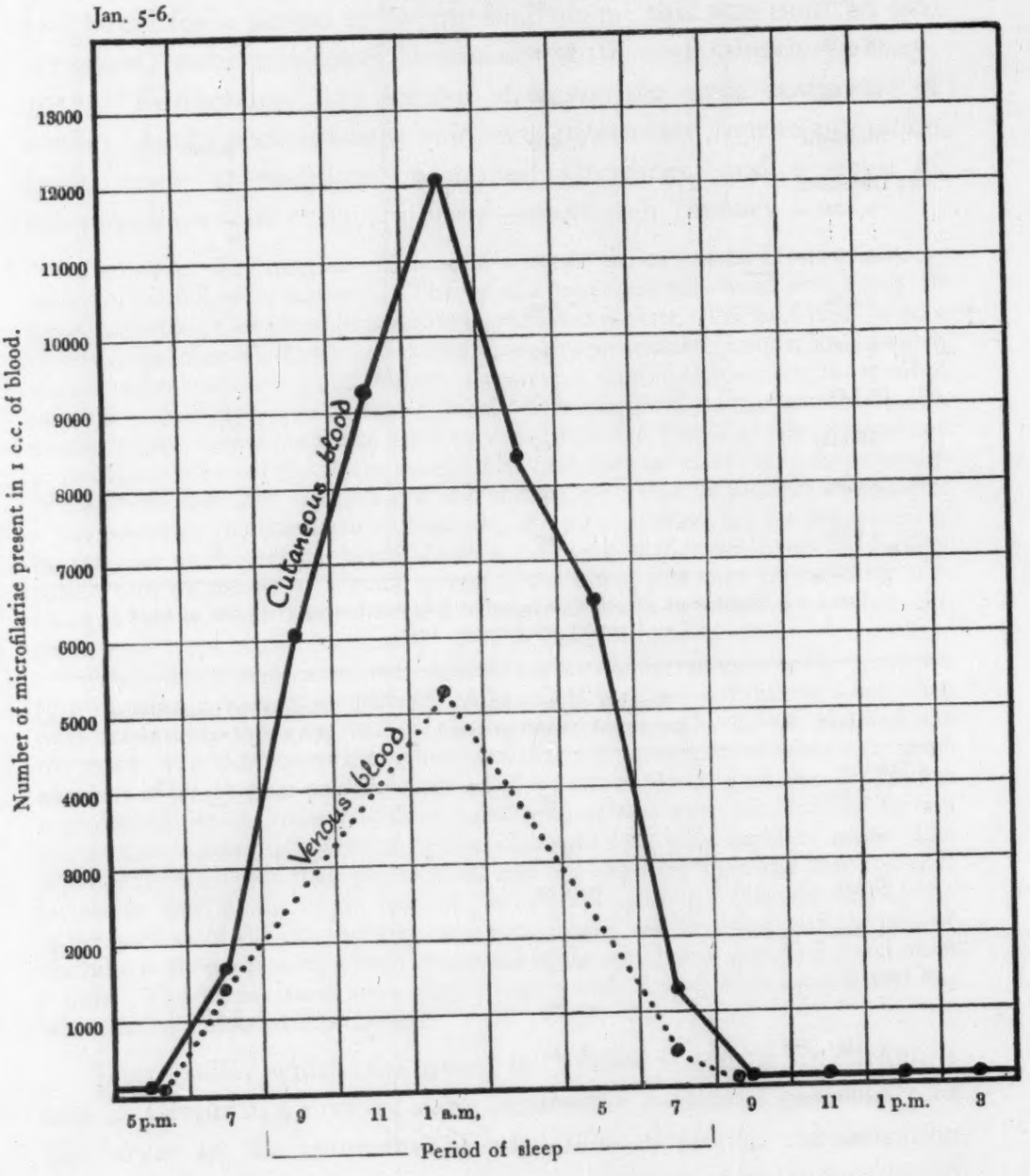
TABLE 1.—Number of microfilariae found at different times during the 24-hour period 21-22 December, 1916.

Hour	Number of microfilariae per c.c. of cutaneous blood	Number of microfilariae per c.c. of venous blood
21 December		
6 p.m. ...	500	—
8 p.m. ...	6,000	—
10 p.m. ...	12,400	—
Midnight ...	12,850	4,000
22 December		
2 a.m.	6,900	—
4 a.m.	6,350	—
6 a.m.	7,700	—
8 a.m.	50	—
10 a.m.	100	—
Midday ...	50	—
2 p.m. ...	50	—
4 p.m. ...	100	—

TABLE 2.—Number of microfilariae found at different times during the 24-hour period 5-6 January, 1917.

Hour	Number of microfilariae per c.c. of cutaneous blood	Number of microfilariae per c.c. of venous blood
5 January		
5 p.m. ...	100	—
7 p.m. ...	1,700	1,600
9 p.m.	6,050	—
11 p.m. ...	9,250	—
6 January		
1 a.m.	12,100	5,250
3 a.m.	8,400	—
5 a.m.	6,400	—
7 a.m.	1,350	550
9 a.m.	110	—
11 a.m.	70	—
1 p.m. ...	90	10
3 p.m. ...	120	—

GRAPH 1.—Showing the periodic variations in number of microfilariae in the cutaneous and venous blood during a 24-hour period.



The figures obtained for the venous blood are very instructive. A single observation was made during the twenty-four hours 21st-22nd December; at midnight when 12,850 larvae were found per cubic centimetre of cutaneous blood, only 4,000 were discovered per cubic centimetre of venous blood. During the twenty-four hours 5th-6th January, a determination of the larvae in the venous blood was made every six hours. The results obtained, together with the corresponding figures for the cutaneous blood, are given in Table 3.

TABLE 3.—Comparing the number of microfilariae per c.c. of venous and cutaneous blood.

Source of Blood	Number of microfilariae per c.c. of blood			
	5 January		6 January	
	7 p.m.	1 a.m.	7 a.m.	1 p.m.
Cutaneous	1,700	12,100	1,350	90
Venous	1,600	5,250	550	10

Two facts are at once apparent from a glance at this table; firstly that the concentration of larvae in the cutaneous blood was on each occasion greater than that in the venous blood, and secondly that the number of larvae per cubic centimetre of venous blood varied in a manner comparable to that in the cutaneous blood.

That the figures for the cutaneous blood were on each occasion higher than the corresponding figures for the venous blood seems to admit of one explanation only, namely that the microfilariae were to some extent—which apparently varied at different times of the day and night—held up in their passage through the cutaneous vessels. In the early evening (7 p.m.) this obstruction appears to be but slight, because there were at that time almost as many larvae in the venous as in the cutaneous blood. It follows from this that obstruction in the cutaneous vessels did not play the most important part in causing the increase in number of the larvae which commenced about this time. Had this increase been due in the main to obstruction in the cutaneous vessels, then the number escaping into the veins should have decreased instead of increasing. As this did not occur, but, on the contrary, the number of larvae in the venous blood

increased almost to the same extent as in the cutaneous blood, it follows that the rapid increase in the cutaneous blood is due for the most part not to any obstruction in this region, but to a sudden rise in the number of larvae in the arterial vessels which supply the skin.

The time of greatest concentration of larvae in the cutaneous blood was also that of greatest concentration in the venous blood. There was, however, at this time a great difference in the degree of concentration in the blood from the two sources, e.g., at midnight, 22nd-23rd December, the cutaneous blood contained 12,850 larvae per cubic centimetre, and the venous blood only 4,000, and at 1 a.m., 6th January, the figures were respectively 12,100 and 5,250. These observations show, firstly, that the relative obstruction to the passage of larvae through the cutaneous vessels is at midnight, or 1 a.m., considerably greater than at 7 p.m.; and secondly, since between 7 p.m. and 1 a.m. the concentration of larvae in both the cutaneous and the venous blood constantly increased, that the number of larvae reaching the cutaneous vessels through the arteries also increased up to midnight or 1 a.m. We were for obvious reasons unable to determine directly the concentration of larvae in the arterial blood, but as the concentration in both cutaneous and venous blood steadily increased from 7 p.m. to 1 a.m., we can infer that the number of larvae per cubic centimetre of arterial blood must at any time during this period have been more than that present in the venous blood, but less than that in the cutaneous blood.

After 1 a.m. the piling up of larvae in the cutaneous blood ceased, and from this time onwards there was a continual fall in concentration, both in the cutaneous and in the venous blood, until at 7 a.m. the figures were respectively 1,350 and 550 per cubic centimetre of blood. We have unfortunately no data to show whether the decrease in concentration of larvae in the venous blood commenced at precisely the same time as in the cutaneous blood, but whether this was the case or not, it is clear that a great decrease had occurred in the venous blood between 1 and 7 a.m. We infer that at 7 a.m. there was still considerable obstruction to the passage of larvae through the cutaneous vessels, and furthermore, as the concentration of larvae in both cutaneous and venous blood fell continuously throughout the period under consideration, that the number of larvae per cubic centimetre of arterial blood at any time during this period must

have been less than that in the venous blood; in other words, that the falling off in the number of microfilariae in the cutaneous blood which commenced shortly after 1 a.m. was due to a sudden decrease in the numbers in the arterial blood.

From 9 a.m. to 5 p.m. the larvae, although constantly found in the cutaneous blood were present in small numbers only (about 100 per cubic centimetre of blood). At 1 p.m. the cutaneous blood contained 90 microfilariae per cubic centimetre, whereas in the venous blood only 10 per cubic centimetre were found. From this we conclude that even when the number of larvae in the cutaneous blood had reached its (almost constant) lowest level, there was still definite obstruction to the passage of the larvae through the cutaneous vessels. During this period (9 a.m. to 5 p.m.), as the number of larvae in the cutaneous blood remained practically constant, it follows that the concentration in the arterial blood was approximately the same as that in the venous blood.

Consideration of these observations as a whole leads us to the conclusion that the nocturnal periodicity of *Microfilaria nocturna* is primarily dependent upon periodic variations in the arterial supply of larvae to the cutaneous vessels. That obstruction to the passage of the larvae through the cutaneous vessels plays no essential part in causing the periodicity is shown by the observations on the cutaneous and venous blood made at 7 p.m. Obstruction to the passage of the larvae through the cutaneous vessels undoubtedly aids in the piling up of larvae in these vessels, but this factor operates at all hours of the day and night and is in no way responsible for the nocturnal periodicity.

REVERSIBILITY OF THE PERIOD OF CUTANEOUS IMMIGRATION

That a nocturnal emigration of the microfilariae to the cutaneous vessels was observed in this patient, who had contracted the infection in Australia, is of itself evidence of the reversibility of the phenomenon. During his journey from Australia to England the patient gradually reversed his hours of sleep and activity, and

coincidently with this a reversal of the period of cutaneous immigration of the microfilariae occurred.

On 29th January the patient was kept up until midnight; the next and subsequent days he was kept in bed from 7 a.m. until 6 p.m., and allowed up during the nights from 6 p.m. until 7 a.m. The patient was placed in a darkened side ward, with the result that he slept undisturbed throughout the whole day. During the night time he carried on his ordinary daily activities and was not allowed to sleep. His meals were served at the following hours:—Breakfast, 6 p.m.; dinner, midnight; tea, 4 a.m.; supper, 6 a.m. While sleep and activity were reversed in this manner, two-hourly enumerations of larvae in the cutaneous blood were made during two 24-hour periods—the first on 2nd-3rd February, and the second on 9th-10th February. The results are shown in Tables 4 and 5, and in Graph 2, Curves 2 and 3. The three curves given in this Graph show clearly the influence of reversing the periods of sleep and activity upon the cutaneous migration of the microfilariae. In each a well-marked periodic cutaneous immigration is evident, but it occurred at different times on the three occasions. In the first (21st-22nd December), when the patient was living under normal conditions as regards sleep and activity, cutaneous immigration occurred from 7 p.m. to 7 a.m.—maximum at midnight; in the second (2nd-3rd February), when reversal of sleep and activity had lasted for four days, it was from 1 a.m. to 1 p.m.—maximum at 6 a.m.; in the third (9th-10th February), when reversal had continued for eleven days, it was from 5 a.m. to 5 p.m.—maximum at midday. In other words, the change from nocturnal to diurnal periodicity, which resulted from reversing the periods of sleep and activity, took place gradually and was not of sudden occurrence; after four days the time of maximum concentration of larvae in the cutaneous vessels had only been set back 6 hours (from midnight until 6 a.m.), whereas after eleven days it had been set back a whole twelve hours (from midnight to midday).

The second curve is particularly interesting in two respects. In the first place cutaneous immigration commenced about 1 a.m., that is, six hours before the patient's bedtime. The result was that the maximum concentration of larvae in the cutaneous vessels was reached an hour before the patient went to bed. In the second

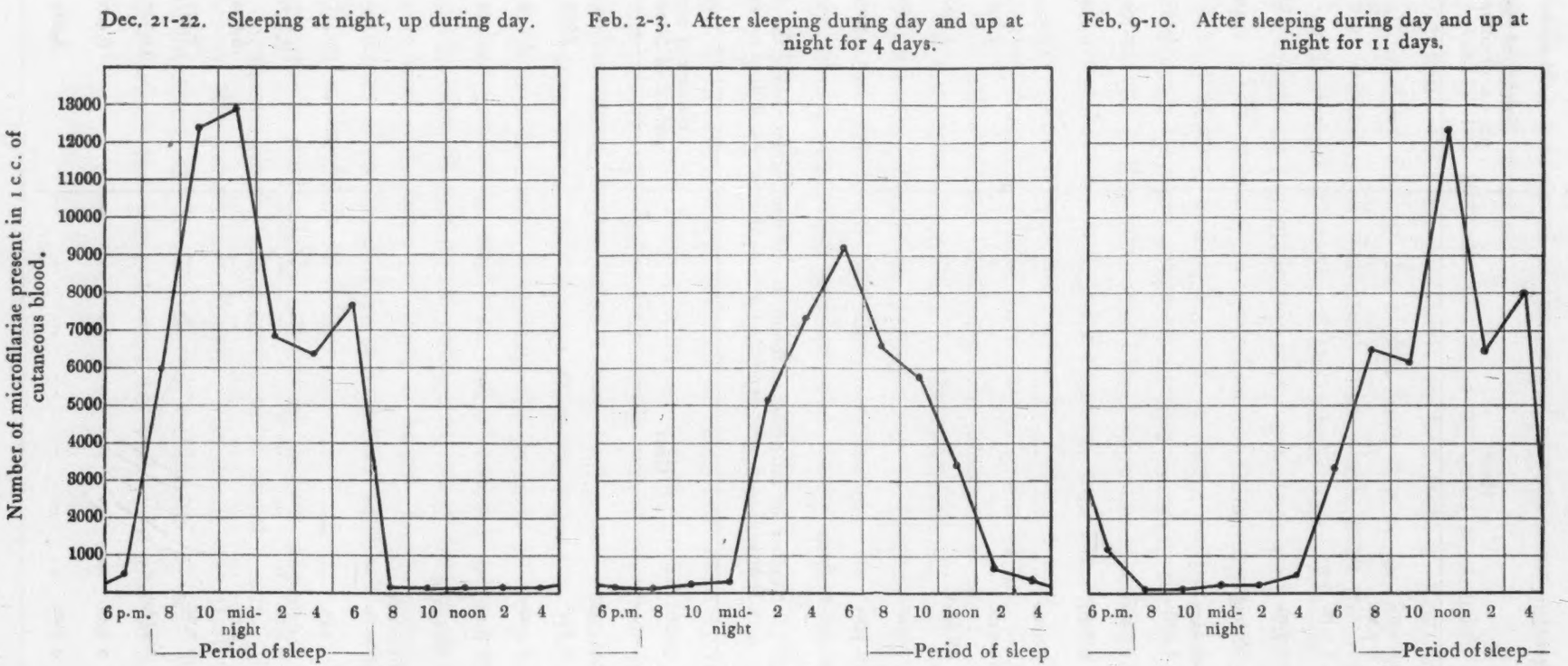
TABLE 4.—Number of microfilariae found at different times during the 24-hour period 2-3 February, 1917, after reversal of periods of activity and sleep for four days.

Hour	Number of microfilariae per c.c. of cutaneous blood
2 February	
6 p.m.	100
8 p.m.	0
10 p.m.	250
Midnight	350
3 February	
2 a.m.	5,150
4 a.m.	7,300
6 a.m.	9,100
8 a.m.	6,600
10 a.m.	5,800
Midday	3,400
2 p.m.	650
4 p.m.	400

TABLE 5.—Number of microfilariae found at different times during the 24-hour period 9-10 February, 1917, after reversal of periods of activity and sleep for eleven days.

Hour	Number of microfilariae per c.c. of cutaneous blood
9 February	
6 p.m.	1,250
8 p.m.	0
10 p.m.	50
Midnight	200
10 February	
2 a.m.	150
4 a.m.	550
6 a.m.	3,350
8 a.m.	6,600
10 a.m.	6,150
Midday	12,300
2 p.m.	6,500
4 p.m.	8,000

GRAPH 2.—Showing the periodic variations in number of microfilariae in the cutaneous blood during 24-hour periods before and after reversal of the periods of sleep and activity.



place the maximum number of larvae found per cubic centimetre of cutaneous blood was in this instance distinctly less than that shown in Curves 1 and 3—the figures are respectively 12,850, 9,100 and 12,300. This lower maximum may find its explanation in an observation referred to earlier in this paper. At 7 p.m. (5th January), an hour before bedtime, the numbers of larvae per cubic centimetre of cutaneous and venous blood were respectively 1,700 and 1,600. This observation, taken in conjunction with the others made at different times on the same date (see Table 3) shows that just before bedtime, when presumably fatigue is at its height, the microfilariae experience least obstruction in their passage through the skin. The lower maximum concentration (9,100) shown in the second curve in Graph 2 was obtained just before bedtime. There was on this occasion less piling up of larvae in the cutaneous vessels, possibly owing to diminished obstruction, due to the relaxation of fatigue, than on the other two occasions (Curves 1 and 3, Graph 2), when the maximum concentration occurred about the middle of the period of sleep.

Before leaving this subject, we might draw attention to the interesting fact that during the period of observation (21st December, 1916, to 10th February, 1917) the number of microfilariae circulating through the cutaneous blood, as judged by the maximum concentration, remained at an almost constant level (Table 6).

TABLE 6.—Showing the number of larvae per cubic centimetre of cutaneous blood at the hour of greatest concentration.

Date	Hour of maximum concentration of larvae in cutaneous vessels	Number of microfilariae found per c.c. of cutaneous blood
21-22 December ...	Midnight	12,850
6 January ...	1 a.m.	12,100
3 February ...	6 a.m.	9,100*
10 February ...	Midday	12,300

* The reason for this low figure has already been discussed.

THE URINE

As mentioned in the brief clinical history given at the beginning of the paper, the urinary symptoms were at first intermittent. During the course of the illness ($2\frac{1}{2}$ years) the relapses became more and more prolonged. From October, 1916, to 12th March, 1917, when the patient left hospital, there was no remission.

The appearance of the urine varied; at times it was like cream, at others like watered milk, more rarely it was only faintly turbid. As a rule it contained more or less blood, which on standing settled to the bottom of the vessel. Not infrequently the whole urine coagulated into a soft jelly having the appearance of a blanc mange; this was preventable by the addition of sodium citrate solution.

When the various specimens passed during a period of 24 hours were compared one with another they were found to exhibit considerable differences in appearance (see Plate III). Mackenzie (1882) records of his patient that certain differences were observed between the urine passed by day and by night; but that the differences were not constant. He writes: 'The night urine is much more milky, does not form so large a coagulum, and contains less blood.'

From 5th January to 12th March we examined each specimen of urine passed; the volume and specific gravity were determined, and a rough comparative estimation made of the opacity and amount of blood contained. It was observed that as a general rule the specimen of urine passed about 6 or 7 a.m. contained the smallest quantity of blood and was least opaque. The urine voided about this time was often almost normal in appearance; this was especially the case if the bladder had been emptied earlier in the morning, thus avoiding mixture of the morning urine with that secreted earlier during the night.

With a view to obtaining more precise information on this subject a series of observations was made, firstly when the patient was living under normal conditions as regards sleep and activity (12th-13th January, 17th-18th January, and 26th-27th January), and secondly when the periods of sleep and activity had been reversed (2nd-3rd February and 9th-10th February). On these occasions the patient was requested to empty his bladder at least once every

three to four hours. The actual amount of blood contained in each specimen of urine was determined by means of a Zeiss comparison spectroscope, according to the method described by Barratt and Yorke (1909). The relative opacity was ascertained by diluting portions of the various specimens with water until they were of the same degree of opacity as an arbitrary standard; the opacity of the individual specimens was then expressed in multiples of this standard. The results which are given in Tables 7, 8 and 9 show that as a general rule at the end of the period of sleep the urine was least opaque and contained the smallest amount of blood.

The explanation appears to be that the opacity, which was presumably due to fat as it almost entirely disappeared after shaking with ether, was caused by the escape of chyle into the urine; at the end of the period of rest (period of starvation) the smallest amount of chyle would be present in the urine. The haematuria, probably due to haemorrhage from lymph varices, would naturally tend to be at its minimum at the end of the period of rest.

PERIODICITY OF MICROFILARIAE IN THE URINE

So far as we are aware, the only reference to this subject is that of Mackenzie, who wrote: 'Filariae were present in both, but probably in greater number in the day than in the night urine.'

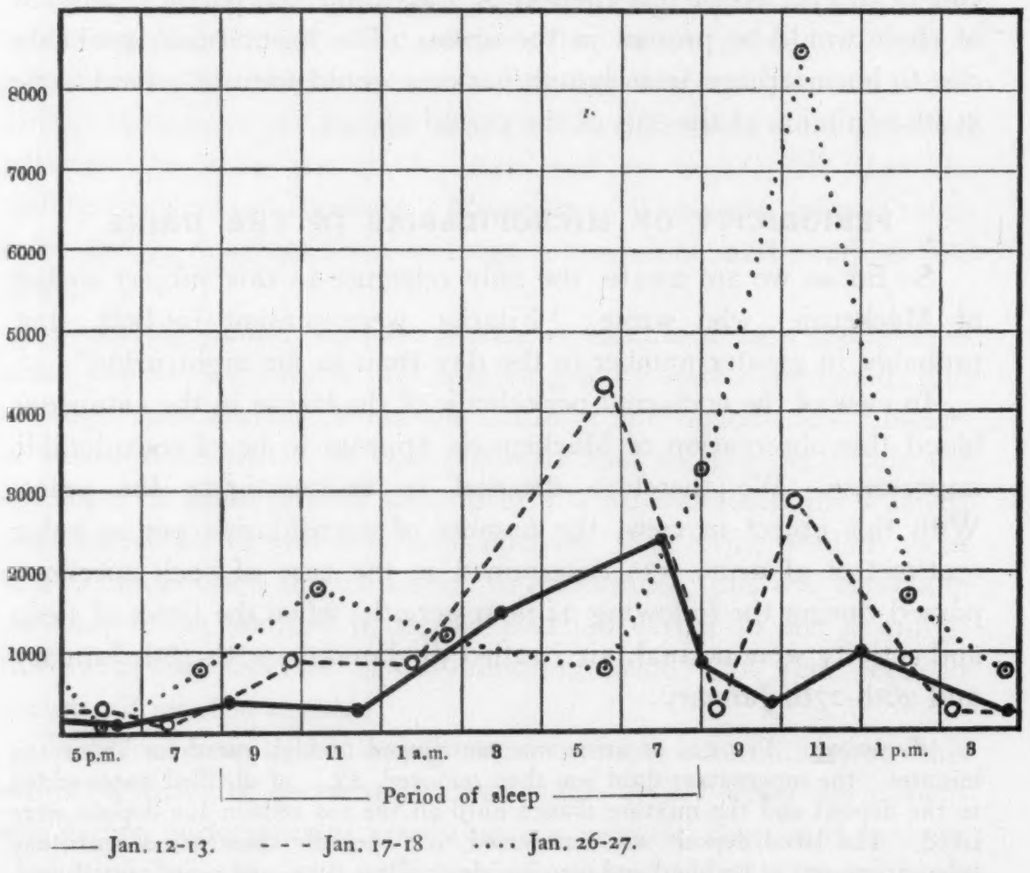
In view of the nocturnal periodicity of the larvae in the cutaneous blood this observation of Mackenzie's appears to be of considerable importance. We therefore decided to re-investigate the point. With this object in view, the number of microfilariae per 10 cubic centimetres of urine was determined in the case of each specimen passed during the following 24-hour periods, when the times of sleep and activity were normal, viz., 12th-13th January, 17th-18th January and 26th-27th January.

Technique. Ten c.c. of urine was centrifuged at high speed for about ten minutes ; the supernatant fluid was then removed, 2 c.c. of distilled water added to the deposit and the mixture shaken until all the red cells in the deposit were laked. The laked deposit was transferred to a specially constructed centrifuge tube, drawn out at its blind end into a wide capillary tube, and again centrifuged. By this procedure the larvae were deposited at the bottom of the capillary portion of the tube below the layer of red cell stromata. The supernatant fluid was removed and the capillary tube cut through near its blind end, but above the lowest level of the red cell stromata. By means of a fine pipette the contents of the blind

end fragment of the capillary tube were removed and a number (usually 4-6 sufficed) of coverslip preparations were made The capillary tube and pipette were washed out with a drop of water and a coverslip preparation made from the washings. As a result of this treatment the microfilariae were found to be motionless, but nevertheless, on account of their high refractility, could easily be distinguished lying amongst the stromata. The total number of microfilariae in the coverslip preparations was counted as in the case of the blood.

The results, which are given in Table 7, show that the number of larvae present in 10 cubic centimetres of urine varied greatly at different times during a 24-hour period. Curves (Graph 3) constructed from the data obtained were very irregular, and gave no indication of either a nocturnal or diurnal periodicity.

GRAPH 3.—Number of microfilariae per 100 c.c. of urine at different times during three 24-hour periods.



In order to arrive at an explanation of this remarkable variation in the number of microfilariae in different specimens passed during the 24-hour period, it is necessary to know something regarding the

TABLE 7.—The results of examination of the various specimens of urine passed during three 24-hour periods when the patient was active during the day and slept at night.

Date and time specimen passed	Volume of urine c.c.	Sp. gr.	Relative opacity	Percentage of blood present	Amount of blood passed c.c.	Number of larvae per 10 c.c. of urine	Total number of larvae present	Number of larvae per 1 c.c. of urinary blood
January 12-13 3.45 p.m. to 5.15 p.m.	465	1004	1	·3	1·4	6	279	200
8.15 p.m.	280	1012	8	·35	·98	34	952	971
11.30 p.m.	395	1007	4	·3	1·18	19	750	633
3.0 a.m.	280	1008	4	·6	1·68	140	3920	2333
6.50 am.	70	1020	trace	·3	·21	235	1645	7833
8.0 a.m.	30	1023	trace	·3	·09	88	264	2933
9.45 a.m.	70	1015	4	1·8	1·26	33	231	183
Noon	160	1019	12	3·8	6·0	101	1616	266
3.45 p.m.	125	1025	3	·7	·87	13	102	186
					13·67		9759	
January 17-18 2.15 p.m. to 5.0 p.m.	110	1020	4	1·3	1·43	26	286	200
6.50 p.m.	150	1015	4	·53	·8	10	150	188
8.0 p.m.	180	1010	8	·75	1·35	41	738	546
9.45 p.m.	125	1012	4	·6	·75	94	1175	1566
1.20 a.m.	290	1009	4	·37	1·07	89	2581	2405
5.40 a.m.	140	1011	2	·76	1·06	429	6006	5644
8.15 a.m.	75	1017	trace	·1	·07	27	202	2700
10.15 a.m.	70	1013	4	·6	·42	195	1365	3250
1.10 p.m.	150	1016	8	3·4	5·1	96	1440	282
2.15 p.m.	205	1010	4	·6	1·23	26	533	433
					13·28		14476	
January 26-27 3.40 p.m. to 5.0 p.m.	100	1014	12	2·8	2·8	8	80	28
6.30 p.m.	275	1007	12	1·0	2·75	17	467	170
7.35 p.m.	75	1017	8	1·3	·97	81	607	623
10.25 p.m.	120	1021	8	1·2	1·44	182	2184	1518
1.50 a.m.	160	1012	4	·3	·48	116	1856	3886
5.20 a.m.	90	1013	1	·1	·09	88	792	8800
8.0 a.m.	55	1021	1	·5	·27	331	1798	6620
10.20 a.m.	70	1000	4	4·0	2·8	851	5957	2127
1.10 p.m.	95	1018	4	2·0	1·9	172	1634	860
3.40 p.m.	175	1014	8	2·2	3·85	75	1312	340
					17·35		16687	

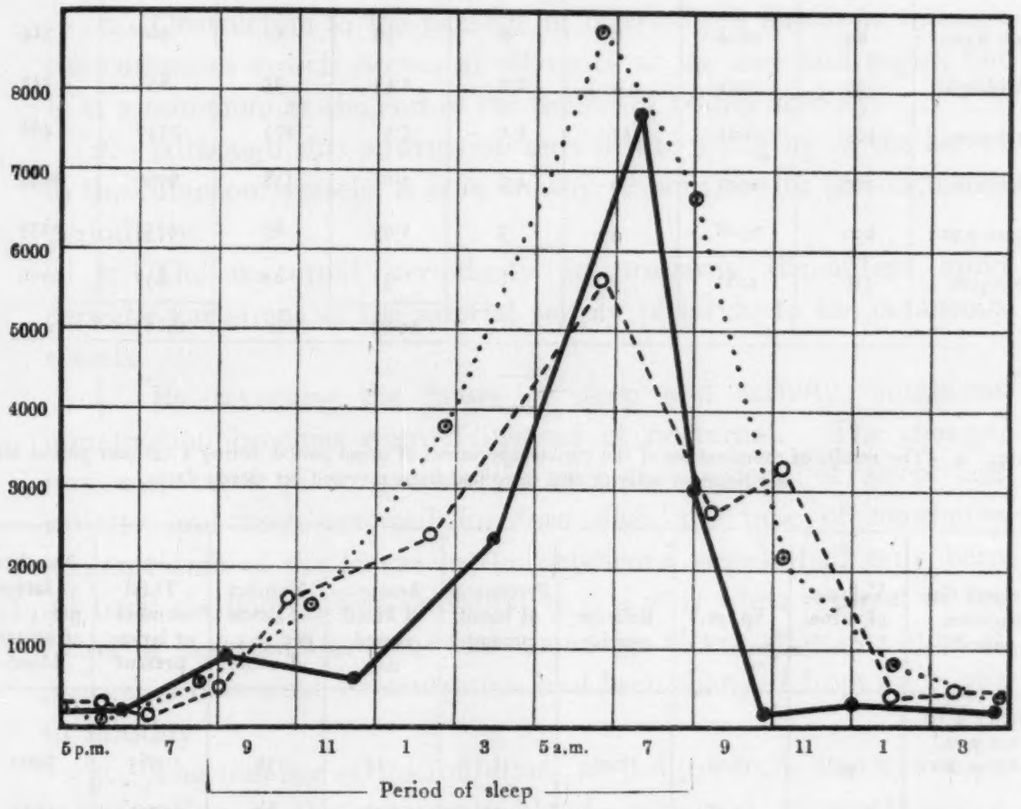
path by which larvae reach the urine. Possibly some of the larvae deposited by the adult worms in the lymphatics, instead of passing by way of the thoracic duct into the blood stream, travel back along the lymphatic vessels and reach the varices in the kidneys or bladder and thence escape into the urine. To what extent this occurs, if at all, we do not know. It is obvious, however, that a considerable number of microfilariae must reach the urine with the blood. Now, having ascertained the number of larvae per 10 cubic centimetres of urine and also the percentage of blood it contains, one can, on the assumption that all the larvae in the urine come from the blood, calculate the number of larvae per cubic centimetre of urinary blood. The data obtained by such calculation are given in Table 7, and in Graph 4.

In striking contrast to the curves (Graph 3) representing the number of larvae per 100 cubic centimetres of urine, these curves (Graph 4) reveal the existence of a regular periodicity corresponding with that of the larvae in the cutaneous blood, with the difference that the maximum concentration occurred several hours later than in the cutaneous blood. Similar observations made when the times of sleep and activity were reversed (2nd-3rd February and 9th-10th February) gave like results (Tables 8 and 9). The number of microfilariae per cubic centimetre of urinary blood changed in a manner strictly comparable with that representing the concentration in the cutaneous blood.

So uniform were the results of our inquiry in this direction that we feel warranted in inferring that the, at first sight unaccountable, variation in the number of larvae in successive specimens of urine during a 24-hour period is to be explained on the grounds that the majority of the microfilariae reach the urine from the blood stream, and, furthermore, that the number of larvae in the renal and vesical vessels exhibits a nocturnal periodicity analagous to that occurring in the cutaneous vessels.

That the curves representing the number of larvae in the urinary blood and those representing the number in the cutaneous blood do not resemble one another still more closely is probably due to technical difficulties in determining exactly the number of larvae in 10 cubic centimetres of urine, and also in ascertaining precisely the percentage of blood it contains. The error in estimating the

GRAPH 4.—Number of microfilariae per c.c. of urinary blood at different times during three 24-hour periods.



—— Jan. 12-13. - - - Jan. 17-18. .... Jan. 26-27.

TABLE 8.—The results of examination of the various specimens of urine passed during a 24-hour period after the times of activity and sleep had been reversed for 4 days.

Date and time specimen passed	Volume of urine c.c.	Sp. gr.	Relative opacity	Percentage of blood present	Amount of blood passed c.c.	Number of larvae per 10 c.c. of urine	Total number of larvae present	Number of larvae per 1 c.c. of urinary blood
February 2-3 2.0 p.m. to 6.0 p.m.	95	1020	trace	·1	·1	24	228	2400
9.0 p.m.	80	1024	2	·7	·56	50	400	714
Midnight	90	1021	4	2·7	2·43	95	855	351
3.0 a.m.	100	1022	12	5·5	5·5	273	2730	496
6.0 a.m.	200	1011	4	1·5	3·0	338	6760	2253
10.20 a.m.	650	1008	4	·3	1·95	67	4355	2233
2.0 p.m.	310	1011	1	·1	·31	27	837	2700
					13·85		16165	

TABLE 9.—The results of examination of the various specimens of urine passed during a 24-hour period after the times of activity and sleep had been reversed for eleven days.

Date and time specimen passed	Volume of urine c.c.	Sp. gr.	Relative opacity	Percentage of blood present	Amount of blood passed c.c.	Number of larvae per 10 c.c. of urine	Total number of larvae present	Number of larvae per 1 c.c. of urinary blood
February 9-10 2.0 p.m. to 6.0 p.m.	145	1020	trace	·1	·14	39	565	3900
10.10 p.m.	430	1008	2	·45	1·93	87	3741	1933
2.0 a.m.	130	1019	12	2·0	2·6	46	598	230
6.0 a.m.	245	1017	12	2·0	4·9	56	1372	280
9.30 a.m.	220	1015	12	·6	1·32	96	2112	1600
2.0 p.m.	130	1017	1	·2	·26	40	520	2000
					11·15		8908	

percentage of blood in the urine is further accentuated by the changes which occur in the spectroscopic appearance of blood which has stood for some time in urine, and also by the fact that intravesical clotting occurred occasionally; extravesical clotting was prevented by the addition of 4 per cent. sodium citrate solution to the urine immediately it was voided.

CONCLUSIONS

1. Obstruction to the passage of *Microfilaria bancrofti* through the cutaneous vessels occurs at all times of the day and night, but is at a minimum at the end of the period of bodily activity.

2. Although this obstruction aids in the piling up of the larvae in the cutaneous vessels, it is in no way responsible for the nocturnal periodicity.

3. The nocturnal periodicity is primarily dependent upon periodic variations in the arterial supply of larvae to the cutaneous vessels.

4. By reversing the hours of sleep and activity, cutaneous immigration becomes diurnal instead of nocturnal. The change, however, takes place gradually; after the periods of sleep and activity had been reversed for four days the time of maximum concentration of the larvae in the cutaneous vessels had only been set back six hours (from midnight to 6 a.m.); when reversal of the hours of sleep and activity had lasted for eleven days the time of maximum cutaneous concentration had been changed from midnight to midday.

5. The number of microfilariae, as judged from the maximum concentration in the cutaneous blood, remained at practically a constant level during the period of observation (21st December, 1916—10th February, 1917).

6. The number of microfilariae present in 100 cubic centimetres of urine varied greatly at different times during a 24-hour period. These variations, which were irregular and gave no indication of either a nocturnal or diurnal periodicity, are to be explained on the assumption that the majority of the microfilariae escaped into the urine with the blood. Graphs depicting the number of larvae per

cubic centimetre of urinary blood reveal the existence of a regular periodicity corresponding to that of the larvae in the cutaneous blood, with the difference that the time of maximum concentration was several hours later.

7. The number of microfilariae in the renal and vesical vessels exhibits a nocturnal periodicity analogous to that in the cutaneous vessels.

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Barratt and Yorke (1909). *Ann. Trop. Med. & Parasitol.*, Vol. III, p. 138.
Mackenzie (1882). *Trans. Path. Soc. of Lond.*, Vol. XXXIII, p. 394.

EXPLANATION OF PLATE III

A tri-colour photograph of the different specimens of urine voided during a 24-hour period, after standing to allow the blood to sediment.

Fig. 1. Urine passed at noon.

Fig. 2. Urine passed at 3.45 p.m.

Fig. 3. Urine passed at 5.15 p.m.

Fig. 4. Urine passed at 8.15 p.m.

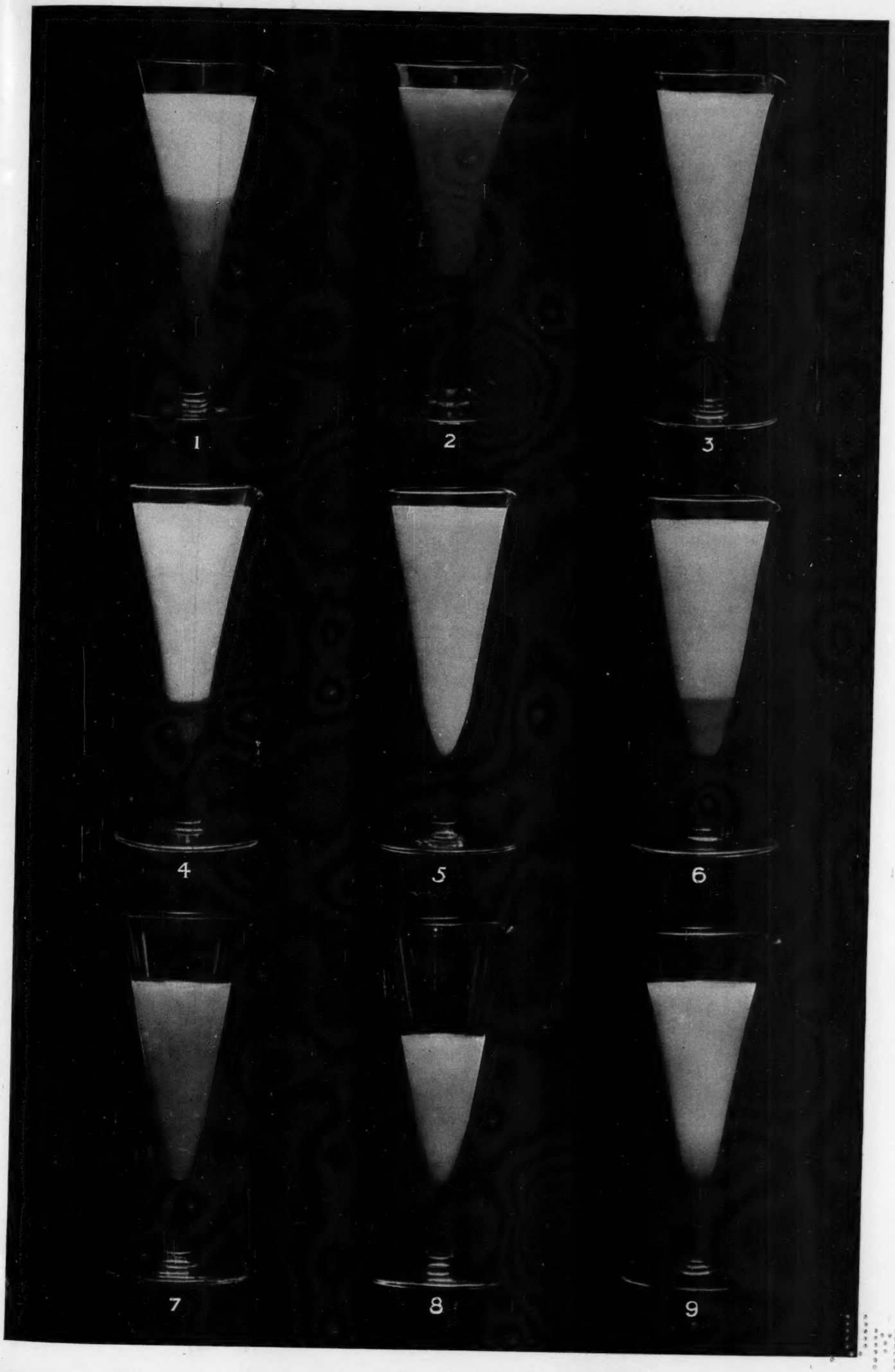
Fig. 5. Urine passed at 11.30 p.m.

Fig. 6. Urine passed at 3.0 a.m.

Fig. 7. Urine passed at 6.50 a.m.

Fig. 8. Urine passed at 8.0 a.m.

Fig. 9. Urine passed at 9.45 a.m.



URINE IN A CASE OF FILARIASIS (*Facing page 69*)

STUDIES IN THE TREATMENT OF MALARIA

III. INTRAVENOUS INJECTIONS OF QUININE BIHYDROCHLORIDE

BY

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Baccelli (1890) was apparently the first to use this method* in the treatment of malaria. He concludes that in very severe cases where other channels of absorption do not suffice, it is the most energetic, most certain, and quickest mode of therapy. This form of treatment has been much used since Baccelli's time, but so far as we are aware, no series of cases has been recorded where the clinical results have been controlled by systematic microscopical examina-

* He used the following formula :—Chininhydrochlorat 1·00 g. ; Chlornatrium 0·75 g.; Destillirtes Wasser 10·00 g.

tions. In this paper we propose to give the results of treating thirty cases of malaria by intravenous injections of quinine bihydrochloride with a view to furnishing these data.

The solution used for injection was a 10 per cent. solution of bihydrochloride of quinine in normal saline. In Baccelli's formula, if correctly given, the strength of the salt solution was 7·5 per cent.

SIMPLE TERTIAN MALARIA (Cases 43-63)

Single injections were given in eight cases. In seven of these the amount of quinine injected was grns. 15, in the other, grns. 10. The detailed results are given in Tables I and II.

In the following temperature charts and tables

Q.V. = intravenous injection of quinine bihydrochloride.
gr. = grains of quinine bihydrochloride.
T. = simple tertian trophozoites or schizonts.
G. = simple tertian gametes.
t. = malignant tertian trophozoites or schizonts.
cr. = malignant tertian gametes.
Neg. = No parasites found.
* = quinine sulphate orally given daily.

The results were similar in all cases. The temperature fell to normal in one to three days, and parasites disappeared from the cutaneous blood in two to five days.

Relapses. Six of the eight cases relapsed. Parasites reappeared in 8-14 days. Febrile paroxysms recurred on an average in 15 days (minimum 10, maximum 20). Of the remaining two cases, one was given quinine orally on the fourth day, and the other died of cerebral haemorrhage of undetermined nature on the tenth day.

As the temperature charts of this series of cases were similar, and presented no points of special interest, two only are reproduced. (Charts 43 and 48).

TABLE I.

Parasitic records after a single intravenous injection of quinine bihydrochloride in simple tertian malaria.

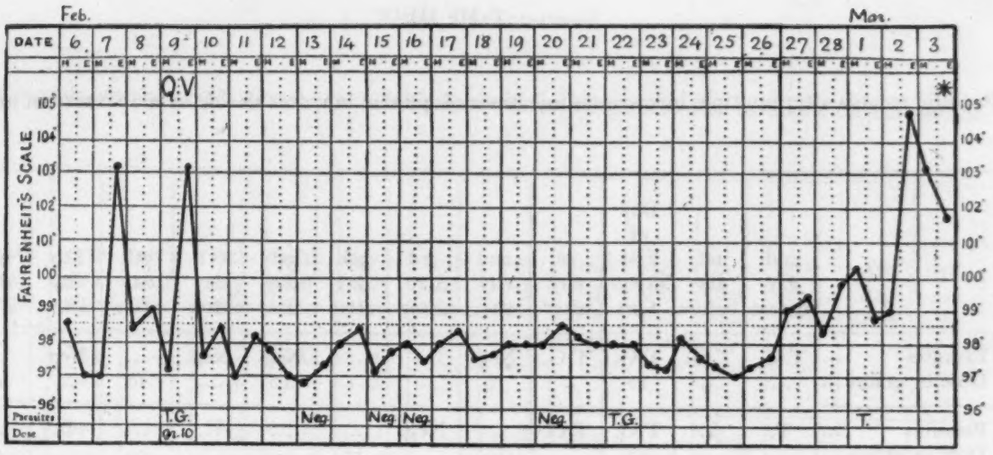
Number of Case		2nd day before	1st day before	Day of injection	1st day after	2nd day after	3rd day after	4th day after	5th day after	6th day after	7th day after	8th day after	9th day after	10th day after	11th day after	12th day after	13th day after	14th day after	15th day after
43	Parasites ...	...	...	T.G.	...	...	...	Neg.	...	Neg.	Neg.	...	...	...	Neg.	...	T.G.	...	...
	Dose in grains ...	...	...	10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
44	Parasites ...	...	...	T.G.	T.G.	...	Neg.	...	...	Neg.	...	Neg.	...	...	...	...	T.	...	T.G.
	Dose in grains ...	...	...	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
45	Parasites ...	...	...	T.G.	T.G.	T.	Neg.	...	...	...	...	T.G.	T.G.	T.G	...	...	...	...	...
	Dose in grains ...	...	...	15	...	...	...	...	...	...	...	...	...	•	...	...	...	...	...
46	Parasites ...	...	T.G.	T.G.	T.	Neg.	Neg.	...	...	...	...	Neg.	...	T.G.	...	...	...	...	...
	Dose in grains ...	...	...	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
47	Parasites ...	T.G.	...	...	T.G.	...	Neg	...	...	...	Neg.	...	...	Neg.	...	...	...	G.	...
	Dose in grains ...	...	...	15	...	...	...	...	...	...	...	...	...	...	...	•	...	...	...
48	Parasites ...	T.G.	...	T.	...	...	Neg.	...	Neg	Neg.	...	T.G.	...	...	...	T.G.	...	...	...
	Dose in grains ...	...	...	15	...	...	...	...	...	...	...	...	...	...	...	...	...	•	...
49	Parasites ...	...	...	T.G.	T.G.	...	G.	...	Neg.	Neg	...	...	...	Died	...	...	...	...	...
	Dose in grains ...	...	...	15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
50	Parasites ...	T.G.	T.G.	T.G.	T.G.	Neg.	...	...	...	...	...	...	...	...	...	...	...	...	...
	Dose in grains ...	...	...	15	...	...	...	...	•	...	...	...	...	...	...	...	...	..	...

TABLE II.

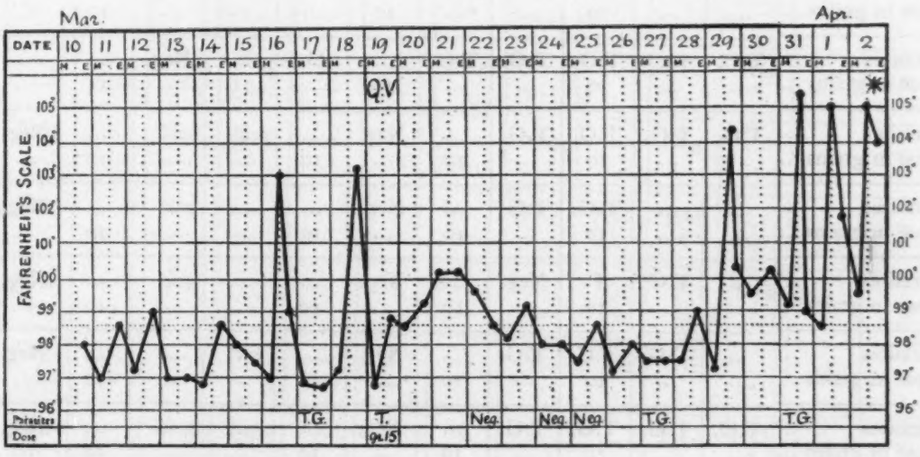
Summary of results of a single intravenous injection of quinine bihydrochloride in simple tertian malaria.

Number of case	Temperature fell to normal in — days after injection	Parasites disappeared from cutaneous blood in — days after injection	Parasitic relapse occurred in — days after injection	Febrile relapse (above 100° F.) occurred in — days after injection	Remarks
43	1	1 - 4	12 - 13	20	—
44	1	2 - 3	9 - 13	16	—
45	1	3	4 - 8	—	Quinine sulphate orally on ninth day
46	1	2	9 - 10	10	—
47	same day	2 - 3	11 - 14	20	—
48	3	1 - 3	7 - 8	10	—
49	—	4 - 5	—	—	Died of cerebral haemorrhage on tenth day after injection
50	1	2	—	—	Quinine sulphate orally on fifth day

CASE 43.



CASE 48.



Multiple injections were given in thirteen cases, 5 in one case and 6 in twelve. The injections were given on Mondays, Wednesdays and Fridays. As a rule grns. 10 were given at each injection, but on a few occasions grns. 15 The detailed results are given in Tables III and IV.

TABLE III.

Parasitic records after multiple intravenous injections of quinine bihydrochloride in simple tertian malaria.

Number of Case		2nd day before	1st day before	Day of first injection	1st day after	2nd day after	3rd day after	4th day after	5th day after	6th day after	7th day after	8th day after	9th day after	10th day after	11th day after
51	Parasites ...	T.G.	T.G.	T.G.	T.G.	Neg.	...	...	Neg.	Neg.	...	Neg	...	...	...
	Dose in grains ...	...	...	10	...	10	...	...	10	...	10	...	10	...	...
52	Parasites ...	T.	...	T.G.	T.G.	...	Neg.	...	...	...	...	(cr.)	...	...	Neg.
	Dose in grains ...	...	...	15	...	10	...	10	...	...	10	...	10	...	10
53	Parasites ...	T.G.	T.G.	T.G.	...	...	...	T.G.	...	Neg.	...	...	...	...	T.
	Dose in grains ...	...	...	10	...	...	10	...	10	...	10	...	...	10	...
54	Parasites ...	T.G.	T.G.	T.G.	T G.	...	...	...	...	T.	...	Neg.	...	...	...
	Dose in grains ...	...	...	10	...	10	...	...	10	...	10	...	10	...	...
55	Parasites ...	...	T.G.	T.G.	T.G.	T.G.	...	T.	...	Neg.	...	...	...	...	Neg.
	Dose in grains ...	...	...	10	...	...	10	...	10	...	10	...	...	10	...
56	Parasites ...	T.G.	...	T.G.	T.G.	T.G.	...	G.	...	Neg.	...	...	...	...	Neg.
	Dose in grains ...	...	...	10	...	...	10	...	15	...	15	...	...	15	...
57	Parasites ...	T.G.	T.G.	T.G.	T.G.	...	Neg.	...	...	...	...	Neg.	...	Neg.	...
	Dose in grains ...	...	...	10	...	10	...	10	...	...	10	...	5	...	10
58	Parasites ...	...	...	T.G.	T.G.	...	Neg.	...	...	...	...	Neg.	...	Neg.	...
	Dose in grains ...	...	...	10	...	10	...	10	...	...	10	...	10	...	10
59	Parasites ...	...	T.G.	T.	Neg.	...	Neg.	...	...	...	...	Neg.	...	Neg.	...
	Dose in grains ...	...	...	10	...	10	...	10	...	...	10	...	10	...	10
60	Parasites ...	...	T.G.	T.G	T.G.	...	Neg	...	...	...	...	Neg.	...	Neg.	...
	Dose in grains ...	...	...	10	...	10	...	10	...	...	10	...	10	...	10
61	Parasites ...	T.G.	T.G.	T.G.	T.G.	...	Neg.	...	...	...	...	Neg.	...	Neg.	...
	Dose in grains ...	...	...	10	...	10	...	10	...	...	10	...	10	...	10
62	Parasites ...	T.G.	T.G.	T.G·	T.G.	...	Neg.	...	...	...	...	Neg.	...	Neg.	...
	Dose in grains ...	...	...	10	...	10	...	10	...	...	10	...	10	...	10
63	Parasites ...	...	...	T.G	T.G.	...	Neg.	...	...	...	...	Neg.	...	Neg.	...
	Dose in grains ...	...	...	10	...	10	...	10	...	...	10	...	10	...	10

TABLE III.—*continued*

Parasitic records after multiple intravenous injections of quinine bihydrochloride in simple tertian malaria—*cont.*

12th day after	13th day after	14th day after	15th day after	16th day after	17th day after	18th day after	19th day after	20th day after	21st day after	22nd day after	23rd day after	24th day after	25th day after	26th day after	27th day after	28th day after	29th day after
...	Neg.	...	Neg.	...	...	T.G.	...	...	...	...	...	...	...	...	...	...	...
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	...	...	(cr.)	...	Neg.	...	...	...	Neg.	Neg.	...	Neg.	...	...	...	...	T.G.
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	T	T.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	Neg.	...	Neg.	...	...	...	...	Neg.	...	T.G	...	...	...	...	...	...	...
10	...	...	...	...	...	...	...	...	...	•	...	...	...	...	...	...	...
...	Neg.	...	Neg.	...	...	Neg.	...	T.	...	...	...	...	...	...	...	...	...
10	...	...	...	...	...	...	...	...	•	...	...	...	...	...	...	...	...
...	Neg.	...	...	...	Neg.	Neg.	...	...	Neg.	...	...	T.	T.G.	...	...	...	...
15	...	...	...	...	...	...	...	...	...	...	...	...	•	...	...	...	...
Neg.	...	...	Neg.	...	Neg.	Neg.	...	...	...	Neg.	...	Neg.	...	...	...	...	Neg.
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	...	...	Neg.	...	Neg.	...	...	...	...	T.G.	T.G.	T.G.	...	...	...	...	...
...	...	...	...	...	...	...	...	...	...	...	...	•	...	...	...	...	...
...	...	...	Neg.	...	Neg.	...	...	...	...	...	T.	T.	T.G.	...	...	...	...
...	...	...	...	...	...	...	...	...	...	...	...	...	•	...	...	...	...
Neg.	...	...	Neg.	...	...	Neg.	...	...	...	T.	...	T.G.	...	...	...	...	...
...	...	...	...	...	...	...	...	...	...	...	...	...	•	...	...	...	...
Neg.	...	...	Neg.	...	Neg.	Neg.	...	...	...	T.	...	T.G.	T.G.	...	...	...	...
...	...	...	...	...	...	...	...	...	...	...	...	...	•	...	...	...	...
Neg.	...	...	Neg.	...	Neg.	...	...	...	...	T.G.	...	...	...	...	...	...	...
...	...	...	...	...	...	...	...	...	...	...	•	...	...	...	...	...	...
Neg.	...	...	Neg.	...	Neg.	Neg.	...	...	...	T.G.	...	T.G	...	...	...	...	...
...	...	...	...	...	...	...	...	...	...	...	...	...	•	...	...	...	...

TABLE IV.

Summary of results of multiple intravenous injections of quinine bihydrochloride in simple tertian malaria.

Number of case	Number of injections	Temperature fell to normal after — injections	Parasites disappeared from cutaneous blood after — injections	Parasitic relapse occurred in — days after first injection	Febrile relapse (above 100° F.) occurred in — days after first injection	Parasitic relapse occurred in — days after last injection	Febrile relapse (above 100° F.) occurred in — days after last injection	Remarks
51	5	2	1	16 - 18	18	7 - 9	9	—
52	6	1	2	25 - 29	29	14 - 18	18	Crescents present during treatment
53	6	1	...	...	14	...	2	Parasites present except on one examination
54	6	3	4	21 - 22	22	9 - 10	10	—
55	6	1	3	19 - 20	...	7 - 8	...	Quinine sulphate 20 gr. orally on 20th day
56	6	1	3	22 - 24	23	10 - 12	11	—
57	6	1	2	...	...	...	...	No relapse in 2½ months
58	6	1	2	18 - 22	22	7 - 11	11	—
59	6	1	1	18 - 23	25	7 - 12	14	—
60	6	1	2	19 - 22	22	8 - 11	11	—
61	6	1	2	19 - 22	25	8 - 11	14	—
62	6	1	2	18 - 22	23	7 - 11	12	—
63	6	1	2	19 - 22	22	8 - 11	11	—

In one case (53) the treatment did not cause the disappearance of parasites from the blood, but as shown in Chart 53 the febrile paroxysms were controlled.

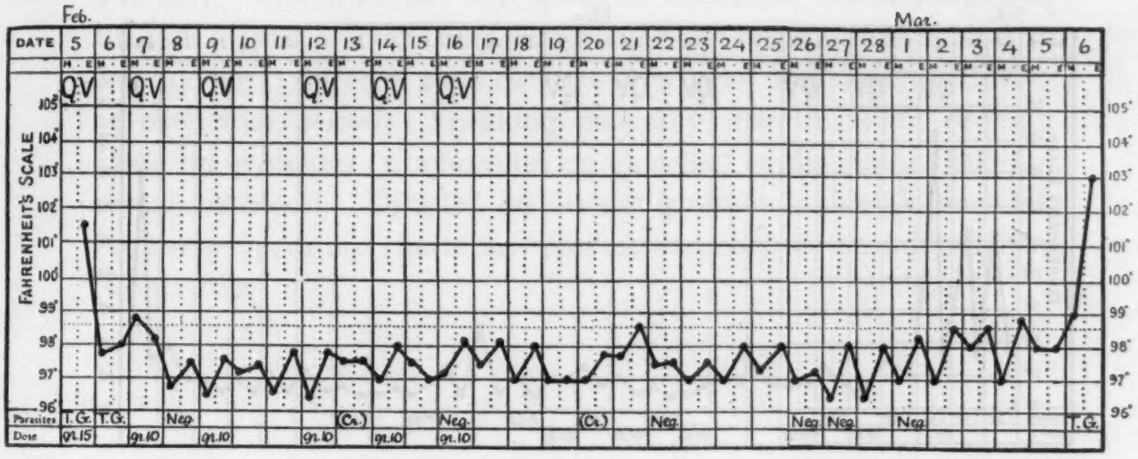
In another case (57) the temperature fell to normal after the first injection, parasites disappeared from the blood after the second injection, and there was no relapse for an observation period of two and a half months.

In the remaining eleven cases the results, which were similar to one another, were as follows: The temperature fell to normal after one to three injections; the parasites disappeared from the blood after one to four injections.

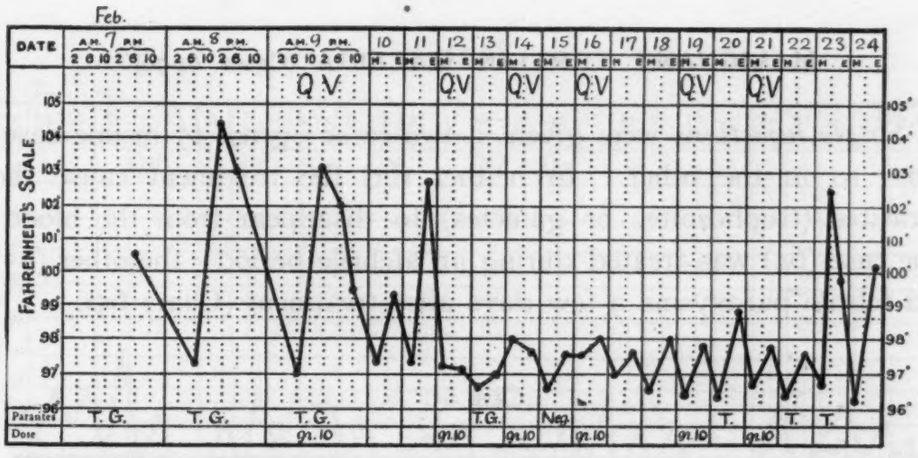
Relapses. All of the eleven cases relapsed. Parasites reappeared in 8-18 days. Febrile paroxysms recurred on an average in 11 days (minimum 11, maximum 15).

As the temperature charts of these eleven cases were similar, three only are reproduced here (Charts 52, 54 and 62).

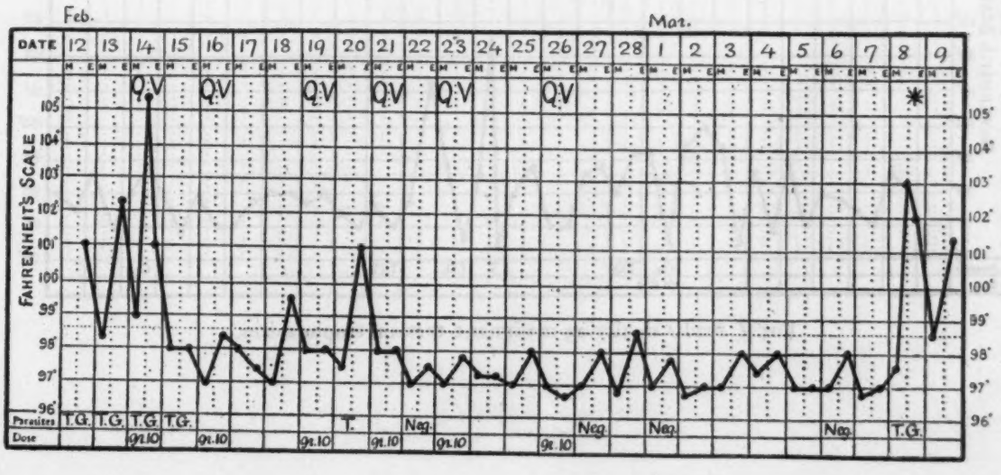
Case 52.



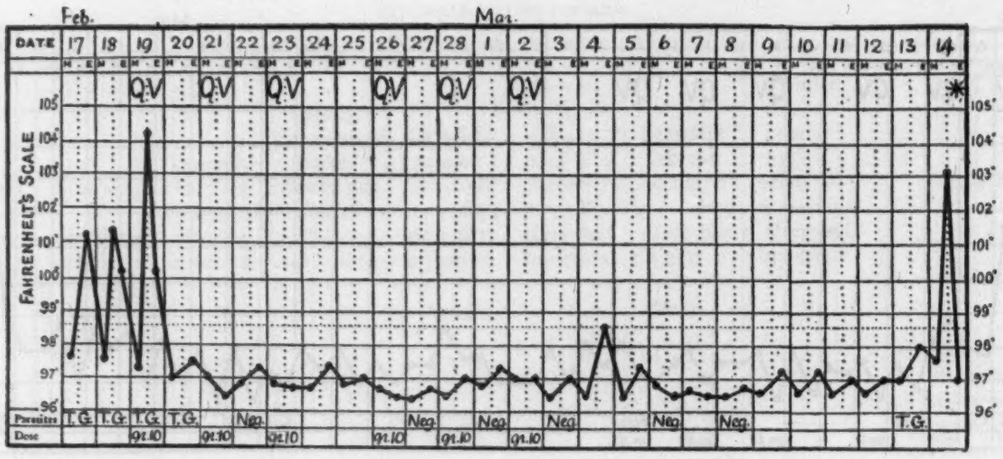
Case 53.



Case 54.



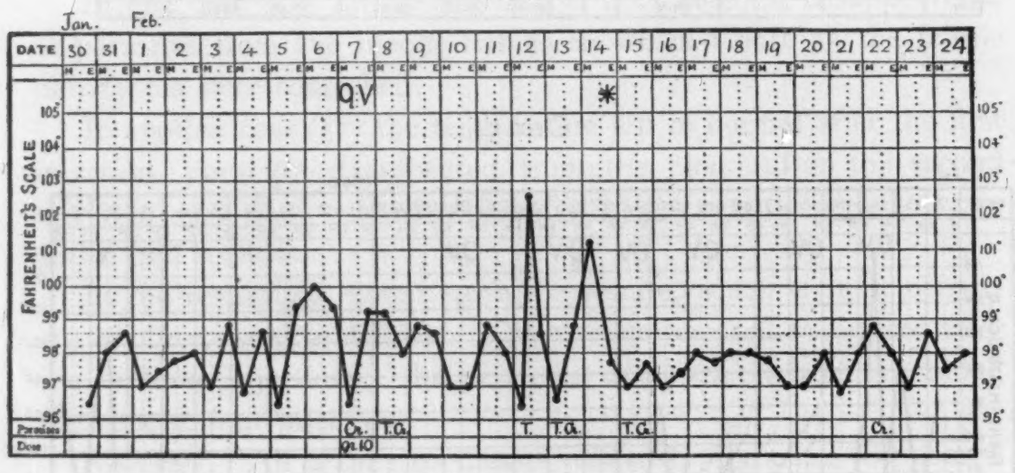
CASE 62.



MALIGNANT TERTIAN MALARIA (Cases 64-70)

Single injections were given in two cases: grns. 15 in one case, grns. 10 in the other. In neither did the treatment cause the parasites (trophozoites or gametes) to disappear from the blood. One case (64) was treated during an afebrile period; the other (65) had febrile paroxysms a few days after treatment (Chart 65).

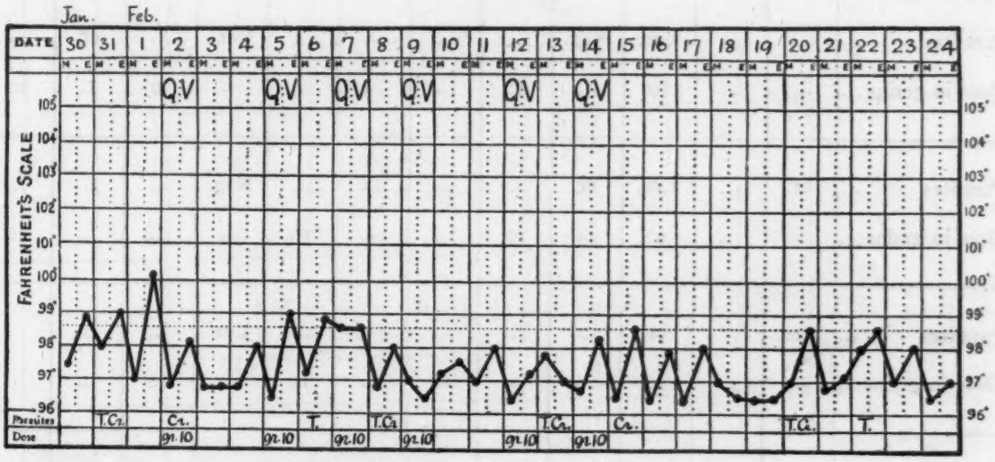
CASE 65.



For *T* read *t* signifying malignant tertian trophozoites.

Multiple injections were given in five cases: 4 in one case, and 6 in four. The injections were given on Mondays, Wednesdays and Fridays; grns. 10 on every occasion. The results were similar to one another. In each case the treatment was administered during an apyrexial period. Speaking generally, the treatment did not cause the parasites (trophozoites or gametes) to disappear from the cutaneous blood. Detailed results are given in Tables V and VI. As the temperature charts are similar, one only is reproduced here (Chart 68).

CASE 68.



For *T* read *t* signifying malignant tertian trophozoites.

TABLE V.

Parasitic records after intravenous injections of quinine bihydrochloride in malignant tertian malaria.

Number of Case		2nd day before	1st day before	Day of first injection	1st day after	2nd day after	3rd day after	4th day after	5th day after	6th day after	7th day after	8th day after	9th day after	10th day after	11th day after
64	Parasites ...	t.	t.	t.	t.	t.	t.	t.cr.	...	...	...	...	...	t.cr.	...
	Dose in grains ...	...	...	15	...	...	...	...	...	...	...	...	...	...	...
65	Parasites ...	...	...	cr.	t.cr.	...	...	...	t.	t.cr.	...	t.cr.	...	...	...
	Dose in grains ...	...	...	10	...	...	...	...	...	...	*	...	...	...	...
66	Parasites ...	...	...	t.cr.	Neg.	...	...	...	...	t.cr.	...	cr.	...	...	...
	Dose in grains ...	...	...	10	...	10	...	...	...	...	10	...	10	...	...
67	Parasites ...	cr.	...	t.cr.	cr.	...	...	...	...	Neg.	...	t.	...	...	...
	Dose in grains ...	...	...	10	...	10	...	...	10	...	...	...	10	...	...
68	Parasites ...	t.cr.	...	cr.	...	...	...	t.	...	t.cr.	...	...	...	...	t.cr.
	Dose in grains ...	...	...	10	...	...	10	...	10	...	10	...	...	10	...
69	Parasites ...	...	...	cr.	Neg.	...	...	...	...	Neg.	...	Neg.	...	...	...
	Dose in grains ...	...	...	10	...	10	...	...	10	...	10	...	10	...	...
70	Parasites ...	t.cr.	...	t.cr.	cr.	...	...	...	...	cr.	...	cr.	...	...	...
	Dose in grains ...	...	...	10	...	10	...	...	10	...	10	...	10	...	...

TABLE V.—*continued.*

Parasitic records after intravenous injections of quinine bihydrochloride in malignant tertian malaria.—*cont.*

12th day after	13th day after	14th day after	15th day after	16th day after	17th day after	18th day after	19th day after	20th day after	21st day after	22nd day after	23rd day after	24th day after	25th day after	26th day after	27th day after	28th day after	29th day after
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	...	...	...	...	...	•	...	...	...	...	...	...	...	...	...	...	...
...	...	...	cr.	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	cr.	...	cr.	...	...	...	...	...	...	Neg.	...	...	...	...	cr.	...	cr.
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	t.cr.	...	t.	...	...	...	...	cr.	...	t.cr.	...	...	...	cr.	cr.	...	cr.
10	...	10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	cr.	...	...	...	...	t.cr.	...	t.	...	...	...	...	t.cr.	...	cr.	...	...
10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	Neg.	...	Neg.	...	...	...	Neg.	Neg.	...	Neg.	...	...	...	...	Neg.	...	Neg.
10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	cr.	...	cr.	...	...	...	...	cr.	...	t.	...	cr.	...	...	cr.	...	Neg.
10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

TABLE VI.

Summary of results of intravenous injections of quinine bihydrochloride in malignant tertian malaria.

Number of case	Number of injections	Remarks
64	1	Treatment during an apyrexial period. Trophozoites and gametes persist
65	1	Fever unaffected : *vide* Chart. Trophozoites and gametes persist
66	4	Treatment during apyrexial period. Gametes persist
67	6	Treatment during apyrexial period. Trophozoites and gametes persist
68	6	Treatment during apyrexial period. Trophozoites and gametes persist
69	6	Treatment during apyrexial period. Gametes disappear, but reappear 29 days after last injection
70	6	Treatment during apyrexial period. Trophozoites disappear, gametes persist

DOUBLE INFECTIONS WITH SIMPLE AND MALIGNANT TERTIAN MALARIA (Cases 71 and 72)

Although some of the cases of simple tertian malaria recorded above showed crescents from time to time, these two cases are grouped separately because at the time of treatment both trophozoites and gametes of *P. vivax* and *P. falciparum* were present.

The detailed results are given in Tables VII and VIII.

TABLE VII.

Summary of results of intravenous injections of quinine bihydrochloride in double infections with simple and malignant tertian malaria.

Number of case	Number of injections	Temperature fell to normal after — injections	Parasites disappeared from cutaneous blood after — injections	Parasitic relapse occurred in — days after first injection	Febrile relapse (above 100° F.) occurred in — days after first injection	Parasitic relapse occurred in — days after last injection	Febrile relapse (above 100° F.) occurred in — days after last injection	Remarks
71	6	2	5	23-24	23	11-12	11	—
72	6	1	2	18	19	6	7	—

TABLE VIII.

Parasitic records after intravenous injections of quinine bihydrochloride in double infections with simple and malignant tertian malaria.

Number of Case		2nd day before	1st day before	Day of first injection	1st day after	2nd day after	3rd day after	4th day after	5th day after	6th day after	7th day after	8th day after	9th day after	10th day after	11th day after
71	*P. vivax* ...	T.G.	T.G.	T.G.	T.G.	T.G.	...	T.G.	...	T.	...	G.	...	...	...
	P. falciparum ...	...	t.cr.	t.cr.	Neg.	Neg.	...	Neg.	...	Neg.	...	Neg.	...	...	...
	Dose in grains ...	...	...	10	...	10	...	...	10	...	10	...	10	...	...
72	*P. vivax* ...	...	T.G.	T.G.	T.G.	T.	...	Neg	...	Neg.	...	...	...	...	Neg.
	P. falciparum ...	...	...	t.cr.	...	cr.	...	Neg.	...	Neg.	...	...	...	...	Neg.
	Dose in grains ...	...	...	10	...	...	10	...	10	...	15	...	...	15	...

TABLE VIII.—*continued.*

Parasitic records after intravenous injections of quinine bihydrochloride in double infections with simple and malignant tertian malaria.—*cont.*

12th day after	13th day after	14th day after	15th day after	16th day after	17th day after	18th day after	19th day after	20th day after	21st day after	22nd day after	23rd day after	24th day after	25th day after	26th day after	27th day after	28th day after	29th day after
...	Neg.	...	Neg.	...	Neg.	...	...	Neg.	...	Neg.	...	Neg.	...	...	T.	T.G.	T.G.
...	Neg.	...	Neg.	...	Neg.	...	...	Neg.	...	Neg.	...	t.	...	...	t.	...	...
10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	Neg.	...	...	...	Neg.	...	...	...	...	...	...	...	...	...	...	...	...
...	Neg.	...	...	...	Neg.	t.	...	t.	...	t.cr.	...	t.cr.	...	t.cr.	t.cr.	...	...
15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

TABLE VI.

Summary of results of intravenous injections of quinine bihydrochloride in malignant tertian malaria.

Number of case	Number of injections	Remarks
64	1	Treatment during an apyrexial period. Trophozoites and gametes persist
65	1	Fever unaffected : *vide* Chart. Trophozoites and gametes persist
66	4	Treatment during apyrexial period. Gametes persist
67	6	Treatment during apyrexial period. Trophozoites and gametes persist
68	6	Treatment during apyrexial period. Trophozoites and gametes persist
69	6	Treatment during apyrexial period. Gametes disappear, but reappear 29 days after last injection
70	6	Treatment during apyrexial period. Trophozoites disappear, gametes persist

DOUBLE INFECTIONS WITH SIMPLE AND MALIGNANT TERTIAN MALARIA (Cases 71 and 72)

Although some of the cases of simple tertian malaria recorded above showed crescents from time to time, these two cases are grouped separately because at the time of treatment both trophozoites and gametes of *P. vivax* and *P. falciparum* were present.

The detailed results are given in Tables VII and VIII.

TABLE VII.

Summary of results of intravenous injections of quinine bihydrochloride in double infections with simple and malignant tertian malaria.

Number of case	Number of injections	Temperature fell to normal after — injections	Parasites disappeared from cutaneous blood after — injections	Parasitic relapse occurred in — days after first injection	Febrile relapse (above 100° F.) occurred in — days after first injection	Parasitic relapse occurred in — days after last injection	Febrile relapse (above 100° F.) occurred in — days after last injection	Remarks
71	6	2	5	23-24	23	11-12	11	—
72	6	1	2	18	19	6	7	—

TABLE VIII.

Parasitic records after intravenous injections of quinine bihydrochloride in double infections with simple and malignant tertian malaria.

Number of Case		2nd day before	1st day before	Day of first injection	1st day after	2nd day after	3rd day after	4th day after	5th day after	6th day after	7th day after	8th day after	9th day after	10th day after	11th day after
71	*P. vivax* ...	T.G.	T.G.	T.G.	T.G.	T.G.	...	T.G.	...	T.	...	G.	...	...	...
	P. falciparum ...	...	t.cr.	t.cr.	Neg.	Neg.	...	Neg.	...	Neg.	...	Neg.	...	...	...
	Dose in grains ...	...	...	10	...	10	...	...	10	...	10	...	10	...	...
72	*P. vivax* ...	...	T.G.	T.G.	T.G.	T.	...	Neg	...	Neg.	...	...	...	...	Neg.
	P. falciparum ...	...	...	t.cr.	...	cr.	...	Neg.	...	Neg.	...	...	...	...	Neg.
	Dose in grains ...	...	...	10	...	...	10	...	10	...	15	...	...	15	...

TABLE VIII.—*continued.*

Parasitic records after intravenous injections of quinine bihydrochloride in double infections with simple and malignant tertian malaria.— *cont.*

12th day after	13th day after	14th day after	15th day after	16th day after	17th day after	18th day after	19th day after	20th day after	21st day after	22nd day after	23rd day after	24th day after	25th day after	26th day after	27th day after	28th day after	29th day after
...	Neg.	...	Neg.	...	Neg.	...	...	Neg.	...	Neg.	...	Neg.	...	...	T.	T.G.	T.G.
...	Neg.	...	Neg.	...	Neg.	...	...	Neg.	...	Neg.	...	t.	...	...	t.	...	...
10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
...	Neg.	...	...	...	Neg.	...	...	...	...	...	...	...	...	...	...	...	...
...	Neg.	...	...	...	Neg.	t.	...	t.	...	t.cr.	...	t.cr.	...	t.cr.	t.cr.	...	...
15	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

EFFECTS OF THE INJECTIONS

In this series of 127 injections, thrombosis occurred in four patients. Two of these had thrombosis in each arm, and two in one arm only. There was no other symptom worthy of note.

CONCLUSIONS

1. In simple tertian malaria, intravenous injections of quinine bihydrochloride in the doses used (grns. 10-15)—either one or a series of six—effect a temporary cure (i.e., cessation of febrile paroxysms and disappearance of parasites from the cutaneous blood). Relapses occur after approximately the same period from the end of treatment, whether one or six injections have been given.

2. In malignant tertian malaria, neither a single nor a series of six injections, in the doses used (grns. 10-15) causes the disappearance of parasites (trophozoites or gametes) from the cutaneous blood.

POSTSCRIPT

Since the above paper was written, an article by Soulié (1917) has appeared, in which he states that, using intravenous injections of quinine-urethane—two injections of 1 gramme each three times a week for a period of four to six weeks—he has effected cures in about sixty cases, relapses being exceptional. The period of observation after cessation of treatment is not given, but it is stated that the cases were followed.

REFERENCES

Baccelli, G. (1890). *Berl. Klin. Woch.*, p. 489.

Soulié, H. (1917). *Bull. Soc. Path Exot.*, Vol. X, p. 217.

STUDIES IN THE TREATMENT OF MALARIA

IV. INTRAMUSCULAR INJECTIONS OF AMYLOPSIN AND TRYPSIN IN SIMPLE TERTIAN MALARIA

BY

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(*Received for publication* 10 *May*, 1917)

Lambelle (1913) stated that he had treated successfully twelve cases of malaria, British soldiers in Upper Burma, by intramuscular injections of the ferments amylopsin and trypsin.

Fretz (1914) records the results of treatment of nineteen cases by this method. He writes: 'Far from being, as I had hoped, of the nature of a *sterilisans magna*, I found that it was in some cases rather a useless and painful operation. It, however, seems undoubtedly to have some effect on the parasites in fresh infections, and better results were obtained in those cases with a longer interval between the injections.'

In view of these conflicting statements we determined to put the treatment to the test. The preparations used by us were Injectio Trypsini and Injectio Amylopsini, Fairchild Bros. and Foster, as recommended by Lambelle. The contents of one ampoule of trypsin and one of amylopsin were taken up in a 10 c.c. syringe, which was then filled with normal saline. The injection was made into the muscle below the spine of the scapula. In every instance the injection was given between 10 and 11 a.m. Where more than one injection was given the interval between the injections was two days.

SIMPLE TERTIAN MALARIA (Cases 73—82)

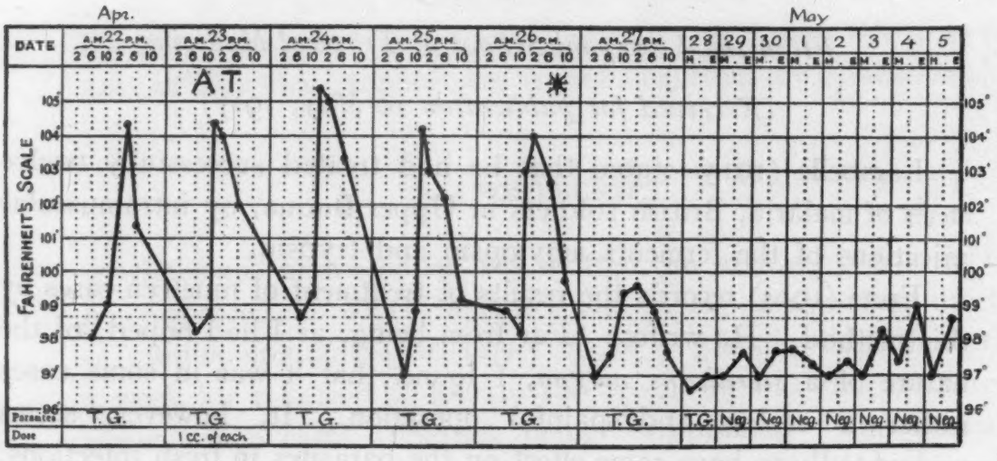
All the cases were adult males infected in Macedonia, at least six months previously, and all had had more or less quinine during this period.

The observations made in the individual cases are recorded in the following charts and table.

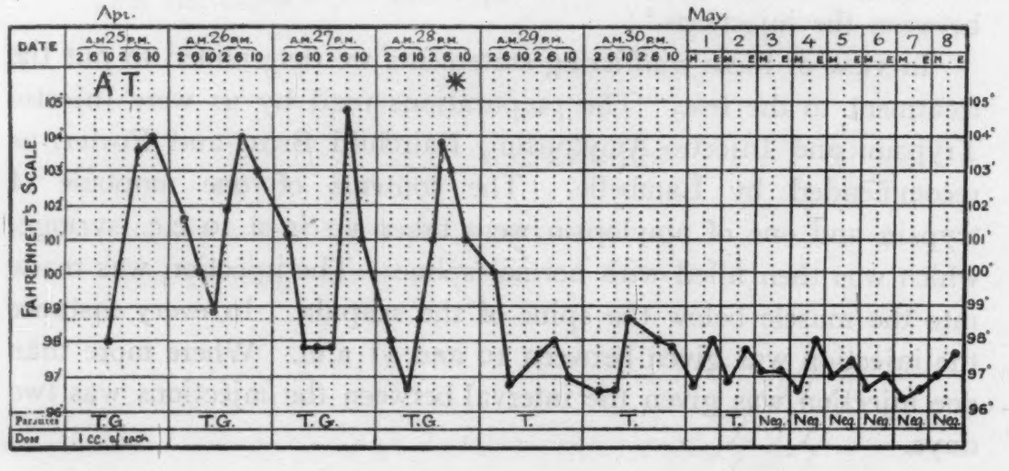
In these charts and table:—

A.T. = intramuscular injection of amylopsin and trypsin.
T. = simple tertian trophozoites or schizonts.
G. = simple tertian gametes.
cr. = malignant tertian gametes.
Neg. = No parasites found.
* = quinine sulphate orally given daily.

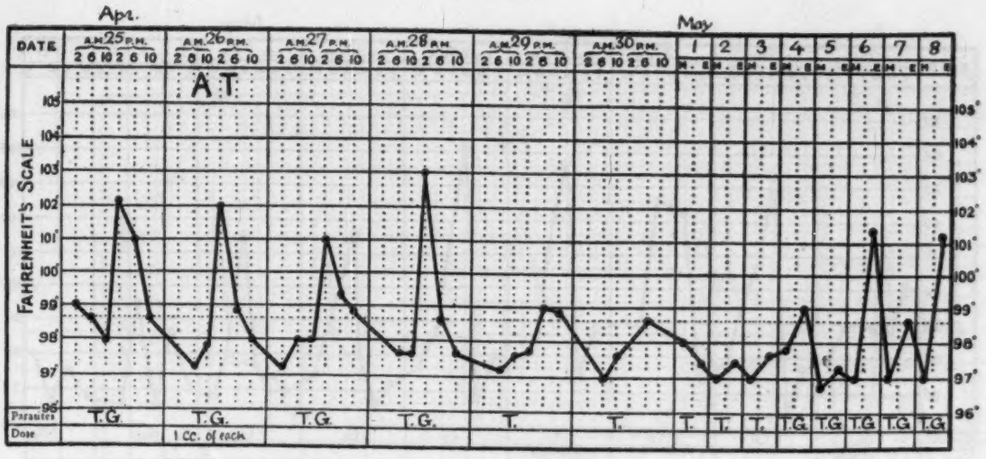
CASE 73.



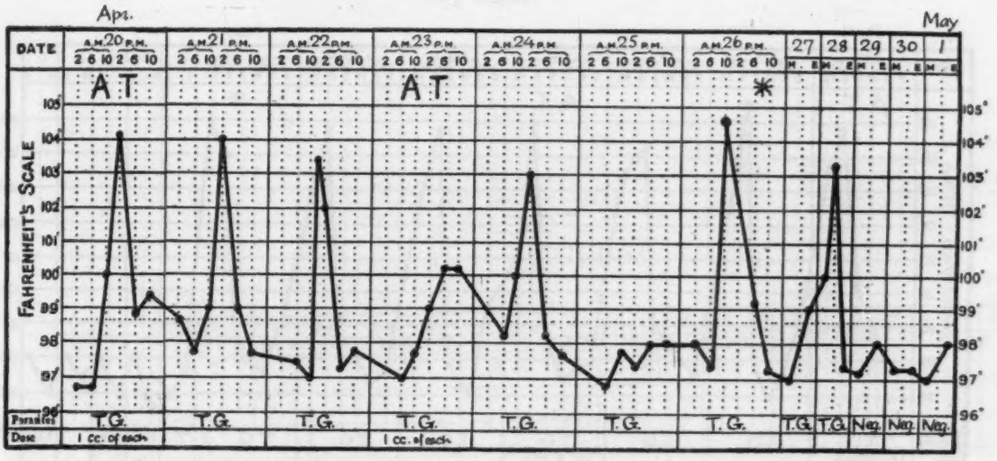
CASE 74.



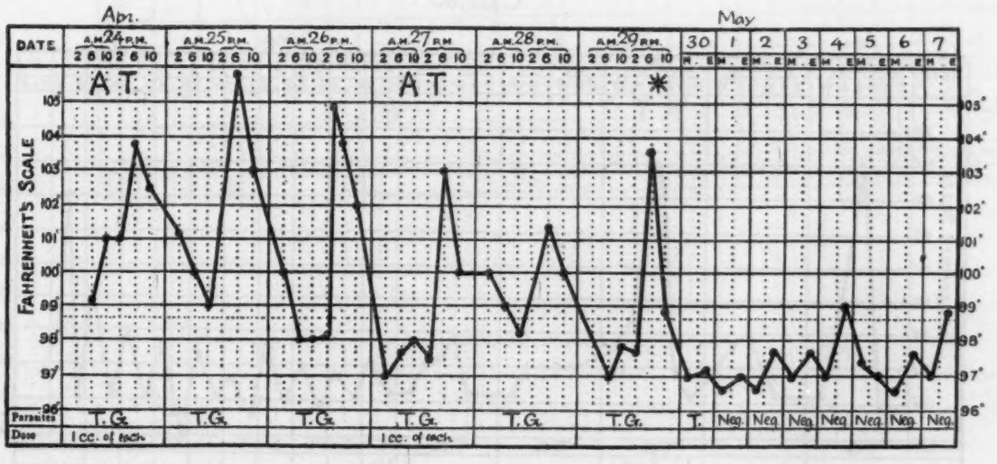
Case 75.



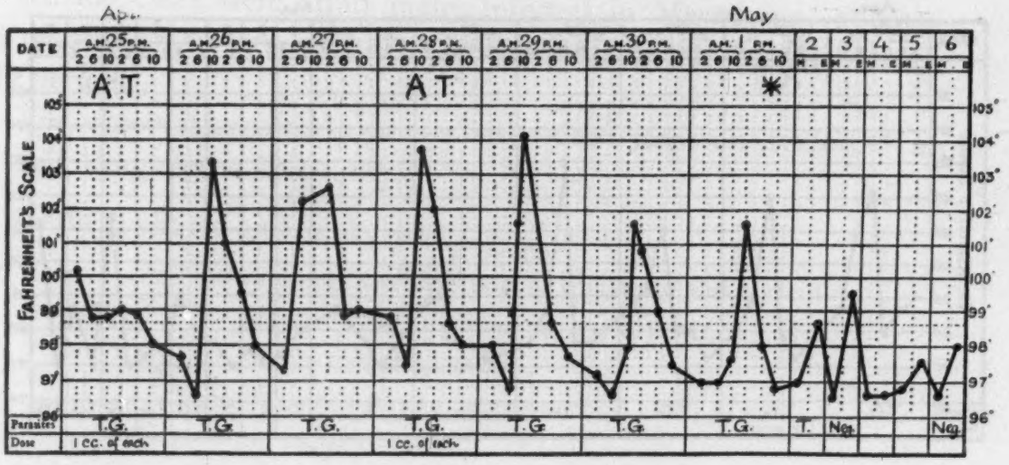
Case 76.



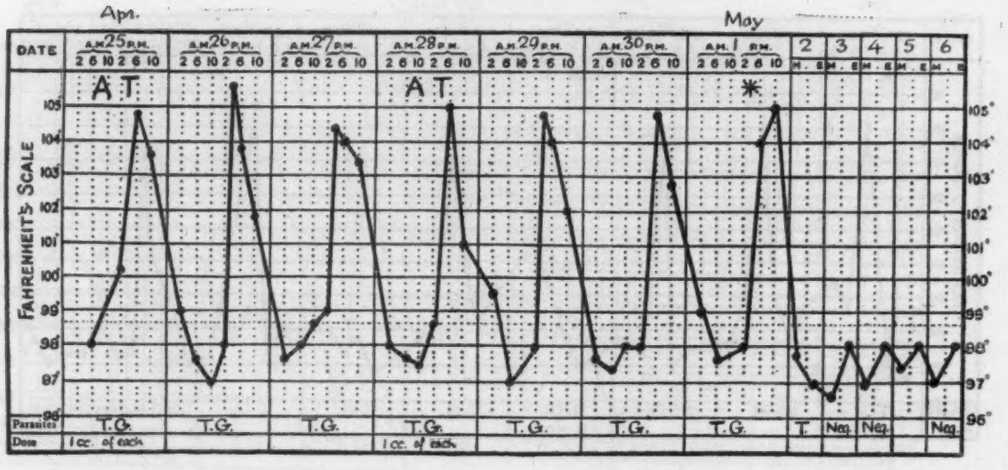
Case 77.



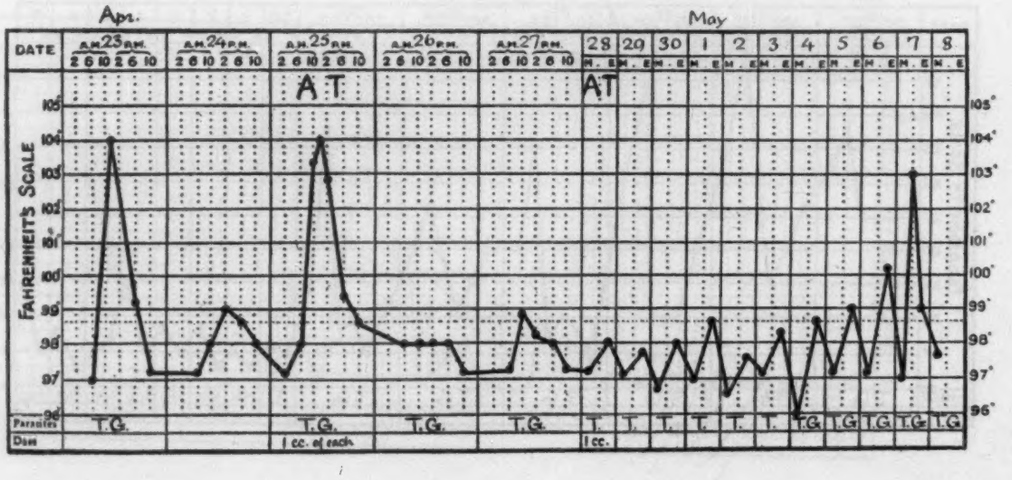
Case 78.



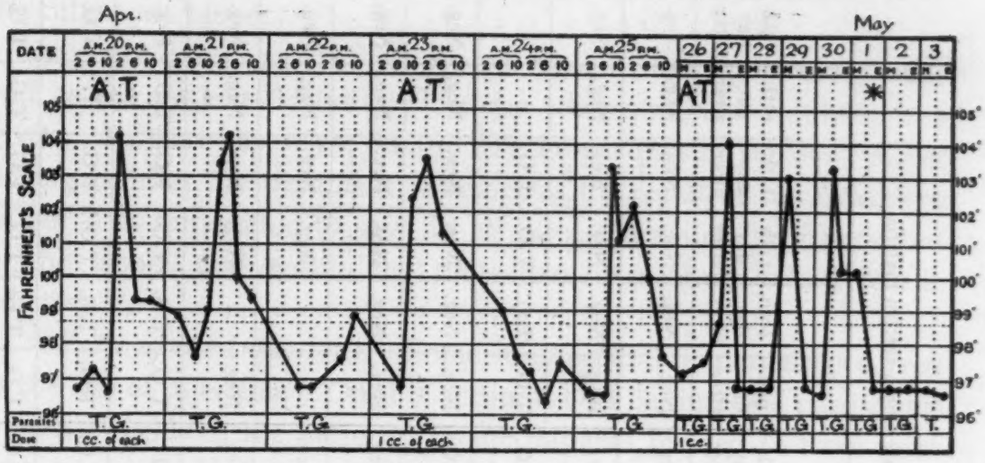
Case 79.



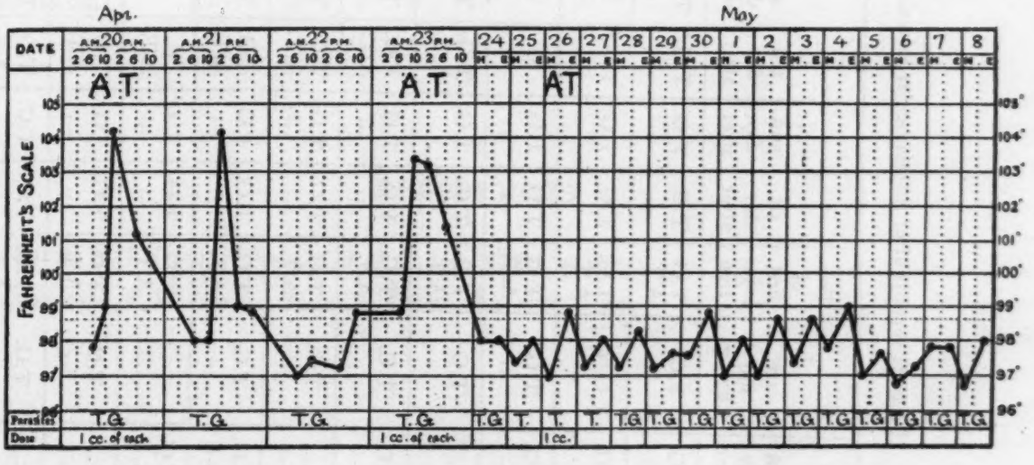
Case 80.



CASE 81.



CASE 82.



TABLE

Parasitic records after intramuscular injections of amylopsin and trypsin in simple tertian malaria.

Number of Case		2nd day before	1st day before	Day of first injection	1st day after	2nd day after	3rd day after	4th day after	5th day after	6th day after	7th day after	8th day after	9th day after	10th day after	11th day after	12th day after	13th day after	14th day after	15th day after
73	Parasites ...	T.G.	T.G.	T.G.	T.G.	T.G.	T.G.*	T.G.	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg	Neg.	Neg.	Neg.
	Injection ...	...	...	A.T.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
74	Parasites ...	T.G.	T.G.(cr.)	T.G.	T.G.	T.G.	T.G.*	T.	T.	...	T.	Neg.	Neg.	Neg.	Neg.	Neg	Neg.	Neg.	...
	Injection ...	...	...	A.T.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
75	Parasites ...	T.G.	T.G.	T.G.	T.G.	T.G.	T.	T.	T.	T.	T.	T.G.	T.G	T.G.	T.G.	T.G.	...	...	...
	Injection ...	...	...	A.T.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
76	Parasites ...	...	T.G.	T.G.	T.G.	T.G.	T.G.	T.G.	T.G	T.G.*	T.G.	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Injection ...	...	...	A.T.	...	...	A.T.	...	...	...	...	...	...	...	...	...	...	...	...
77	Parasites ...	T.G.	T.G.	T.G.	T.G.	T.G.	T.G.	T.G.	T.G.*	T.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	...
	Injection ...	...	...	A.T.	...	...	A.T.	...	...	...	...	...	...	...	...	...	...	...	...
78	Parasites ...	...	T.G.	T.G.	T.G.	T.G.	T.G.	T.G.	T.G.	T.G.*	T.	Neg.	...	...	Neg.	Neg.	Neg.	Neg.	...
	Injection ...	...	...	A.T.	...	...	A T.	...	...	...	...	...	...	...	...	...	...	...	...
79	Parasites	T.G.	T.G.	T.G.	T.G.	T.G.	T.G.	T.G.	T.G.	T.G.*	T.	Neg.	Neg.	...	Neg.	Neg.	Neg.	Neg.	...
	Injection ...	...	...	A.T.	...	...	A.T.	...	...	...	...	...	...	...	...	...	...	...	...
80	Parasites ...	T.G.	...	T.G.	T.G.	T.G	T.	T.	T.	T.	T.	T.	T.G.	T.G.	T.G.	T.G.	T.G.*	Neg.	Neg.
	Injection ...	...	...	A.T.	...	...	A.T.	...	...	...	...	...	...	...	...	...	...	...	...
81	Parasites ...	...	T.G.	T.G.	T.G.	TG.	T.G.	T.G.	T.G.	T.G.	T.G.	T.G.	T.G.	T.G.	T.G.*	T.G.	T.	T.G.	Neg.
	Injection ...	...	...	A.T.	...	...	A.T.	...	...	A.T.	...	...	...	...	...	...	...	...	...
82	Parasites ...	...	T.G.	T.G.	T.G.	T.G.	T.G.	T.G.	T.	T.	T.	T.G.	T.G.	T.G.	T.G.	T.G.	T.G.	T.G.	T.G.
	Injection ...	...	...	A.T.	...	...	A.T.	...	...	A.T.	...	...	...	...	...	...	...	...	...

Blood examinations were made daily in all ten cases. In no instance did the treatment cause the disappearance of parasites from the cutaneous blood.

In seven cases (73, 74, 76, 77, 78, 79 and 81) rigors continued, and as the patients' condition became serious quinine sulphate was given. In three cases (75, 80 and 82) the paroxysms subsided either during or after the treatment, whether as the result of the treatment or not it is impossible to say; in all three, parasites have persisted in the blood, and within ten days two have already had febrile relapses.

Effects of the Injections. Some swelling and tenderness occurred at the site of injection. On this account, and because the paroxysms were not checked, the majority of the patients refused to have more than one or two injections.

CONCLUSION

Intramuscular injections of amylopsin and trypsin proved to be of no value in the treatment of these ten cases of acute simple tertian malaria.

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LAMBELLE, F. W. (1913). *Journ. R.A.M.C.*, Vol. XXI, p. 660.

STUDIES IN THE TREATMENT OF MALARIA

V. INTRAMUSCULAR INJECTIONS OF QUININE ALKALOID IN SIMPLE TERTIAN MALARIA

BY

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(*Received for publication* 13 *July*, 1917)

The fact that soluble salts of quinine however administered are apparently eliminated from the body in a few days, or, at any rate, quinine as such cannot be detected in the urine, led us to try whether the introduction into the body of an insoluble form of quinine, viz., the alkaloid (only very slightly soluble in water) would result in a more gradual absorption, and consequently a more prolonged therapeutic effect.

The solution used by us for this purpose was made in the following way*:—One gramme of quinine alkaloid was dissolved in 1 c.c. of 90 per cent. alcohol, and the volume made up to 3 c.c. with sesame oil. By this means a clear viscid solution capable of being easily injected was obtained, 1 c.c. containing approximately 5 grains of the alkaloid.

* We are indebted to the kind help of Mr. Prosper Marsden in preparing the solution.

SIMPLE TERTIAN MALARIA (Cases 83-120)

All these cases were adult males infected in Macedonia at least nine months previously, and all had had more or less quinine during this period. The observations made on the individual cases are recorded in the charts and tables, pp. 176-182:—

In the following temperature charts and tables:—

Q.A.M. = intramuscular injections of quinine alkaloid.
gr. = grains of quinine alkaloid.
T. = simple tertian trophozoites or schizonts.
G. = simple tertian gametes.
Cr. = malignant tertian gametes.
Neg. = no parasites found.
* = quinine sulphate orally given daily.

Injection of quinine alkaloid brought about:—

(1) A cessation of febrile paroxysms. While the temperature usually fell to normal within four days of the first injection (Chart 102), occasionally where the doses given were larger (30 grains), the temperature did not become normal for six to eleven days (Charts 87, 115, 118 and 119), although the parasites had disappeared in one to two days after the first injection. We are unable to afford any explanation of this febrile reaction with the larger doses.

(2) Disappearance of parasites (trophozoites and gametes) from the cutaneous blood usually in one to two days.

Relapses. Thirty-one of the thirty-eight cases relapsed. Parasites reappeared on an average in 18 days (minimum period 11, maximum 27). Febrile paroxysms recurred on an average in 20 days (minimum 13, maximum 32). The remaining seven cases had not relapsed within an observation period of two months.

A comparison of these results with those recorded by us (1917) with the use of a very soluble salt of quinine, the bihydrochloride, shows that the therapeutic action of the insoluble alkaloid is in no way inferior to that of the soluble salt.

TABLE I.

Summary of results of intramuscular injections of quinine alkaloid in simple tertian malaria.

Number of Case	Dose in grains	Temperature fell to normal in — days after first injection	Parasites disappeared from cutaneous blood in — days after first injection	Parasitic relapse occurred in — days after first injection	Febrile relapse (above 100° F.) occurred in — days after first injection	Remarks
83	1 × 15	1	2	13	13	
84	,,	2	2	22	22	
85	1 × 30	3	2	25	26	
86	,,	2	2	27	32	
87	,,	11	1	22	25	
88	,,	4	1	18	19	
89	,,	same day	1 - 2	17	13	
90	2 × 10	2	3	11	13	
91	,,	1	1	...	...	No relapse in two months
92	,,	1	3	15	19	
93	,,	2	3	15	17	
94	2 × 15	4	2	...	...	No relapse in two months
95	,,	3	2	13	16	
96	,,	4	1 - 2	16	18	
97	,,	2	2	23 - 24	24	
98	,,	3	1	18	20	
99	,,	2	2	14	17	
100	,,	3	3	14	17	
101	,,	1	3	20	21	
102	,,	4	2	24	26	
103	,,	3	3	...	...	No relapse in two months
104	,,	2	2	16	16	
105	,,	1	3	...	...	No relapse in two months
106	,,	3	1	...	...	No relapse in two months
107	,,	3	2	17	15	
108	,,	3	1	15	17	3 c.c. collosal quinine two days before first injection. Crescents on 14th day
109	2 × 20	3	1	25	24	Rigor on 12th day; blood negative
110	,,	3	1	21	26	
111	,,	1	2	19	24	
112	2 × 30	same day	2	22	23	104·4° F. on 3rd day. Crescents on 12th to 16th days
113	,,	Apyrexial period	1	18	23	100·6° F. on 17th day
114	,,	same day	1	16	19	quinine sulphate 15 gr. orally day before first injection
115	,,	7	2	18	19	Crescents on 2nd and 5th days
116	,,	2	2	14	18	
117	,,	3	1	20	24	
118	,,	9	2	15	15	
119	,,	6	2	...	...	100° F. on 16th day. No relapse in two months
120	,,	3	4	...	...	No relapse in two months*

*This case relapsed after discharge from hospital.

TABLE II.

Parasitic records after intramuscular injections of quinine alkaloid in simple tertian malaria.

Number of Case		2nd day before	1st day before	Day of first injection.	1st day after	2nd day after	3rd day after	4th day after	5th day after	6th day after	7th day after	8th day after	9th day after	10th day after	11th day after
83	Parasites ...	...	T.	T.G.	T.G.	Neg.	...	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	15	...	...	...	...	...	...	...	...	...	...	...
84	Parasites ...	...	T.G.	T.	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	15	...	...	...	...	...	...	...	...	...	...	...
85	Parasites ...	...	T.G.	T.G.	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	...	...	Neg.
	Dose in grains ...	...	...	30	...	...	...	...	...	...	...	...	...	...	...
86	Parasites ...	...	...	T.G.	T.G.	Neg.	...	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	...
	Dose in grains ...	...	...	30	...	...	...	...	...	...	...	...	...	...	...
87	Parasites ...	...	T.G.	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	30	...	...	...	...	...	...	...	...	...	...	...
88	Parasites ...	T.G.	...	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	30	...	...	...	...	...	...	...	...	...	...	...
89	Parasites ...	T.G.	T.	G.	...	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	30	...	...	...	...	...	...	...	...	...	...	...
90	Parasites ...	...	...	T.G.	T.G.	G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	T.
	Dose in grains ...	...	...	10	10	...	...	...	...	...	...	...	...	...	...
91	Parasites ...	...	...	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	10	10	...	...	...	...	...	...	...	...	...	...
92	Parasites ...	...	T.G.	T.G.	T.G.	T.G.	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	10	10	...	...	...	...	...	...	...	...	...	...
93	Parasites ...	...	T.G.	T.G.	T.G.	T.G.	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	10	10	...	...	...	...	...	...	...	...	...	...
94	Parasites ...	...	G.	T.G.	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	...	Neg.
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...
95	Parasites ...	...	T.G.	T.G.	G.	Neg.	Neg.	...	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...
96	Parasites ...	...	T.G.	T.G.	...	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...
97	Parasites ...	...	T.G.	T.G.	T.	Neg.	Neg.	Neg.	...	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...
98	Parasites ...	...	...	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...
99	Parasites ...	...	T.G.	T.G.	T.	Neg.	Neg.	Neg.	...	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...
100	Parasites ...	...	T.G.	T.G.	T.G.	G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...
101	Parasites ...	...	T.G.	T.G.	T.G.	T.G.	Neg.	Neg.	Neg.	Neg.	...	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...

TABLE II.—*continued.*

Parasitic records after intramuscular injections of quinine alkaloid in simple tertian malaria.

12th day after	13th day after	14th day after	15th day after	16th day after	17th day after	18th day after	19th day after	20th day after	21st day after	22nd day after	23rd day after	24th day after	25th day after	26th day after	27th day after	Number of Case
Neg. ...	T. ...	T.G. ...	T.G. ...	T.G. ...	T.G. ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	83
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	... ...	Neg. ...	G. ...	T. ...	G. ...	... ...	... ...	... ...	84
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	T. ...	T.G. ...	T.G.* ...	85
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	T. ...	86
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	... ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	T. ...	T. ...	T. ...	T.G. ...	T.G.* ...	T.G. ...	87
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	T. ...	T. ...	... ...	T.G. ...	T.G. ...	... ...	Neg. ...	Neg. ...	... ...	... ...	88
... ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	T. ...	T.G. ...	T.G. ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	89
T.G. ...	T.G. ...	T.G. ...	T.G.* ...	T.G. ...	G. ...	Neg. ...	Neg. ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	90
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	91
Neg. ...	Neg. ...	Neg. ...	T. ...	T. ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	92
Neg. ...	Neg. ...	Neg. ...	T. ...	T. ...	T.G. ...	T.G. ...	T.G.* ...	T.G. ...	T.G. ...	Neg. ...	Neg. ...	... ...	Neg. ...	... ...	... ...	93
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	... ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	94
Neg. ...	T.G. ...	T. ...	T.G. ...	T.G. ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	95
... ...	Neg. ...	Neg. ...	Neg. ...	G. ...	T. ...	T.G. ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	96
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	... ...	T.G. ...	... ...	... ...	... ...	97
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	T. ...	T.G. ...	T.G. ...	T.G. ...	T.G. ...	T.G. ...	... ...	... ...	... ...	... ...	98
Neg. ...	Neg. ...	T. ...	G. ...	T.G. ...	T.G. ...	T.G. ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	99
Neg. ...	Neg. ...	T. ...	Neg. ...	Neg. ...	T. ...	T. ...	T.G. ...	T.G. ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	100
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	T. ...	T.G. ...	... ...	... ...	... ...	... ...	... ...	... ...	101

TABLE II.—*continued.*

Parasitic records after intramuscular injections of quinine alkaloid in simple tertian malaria.

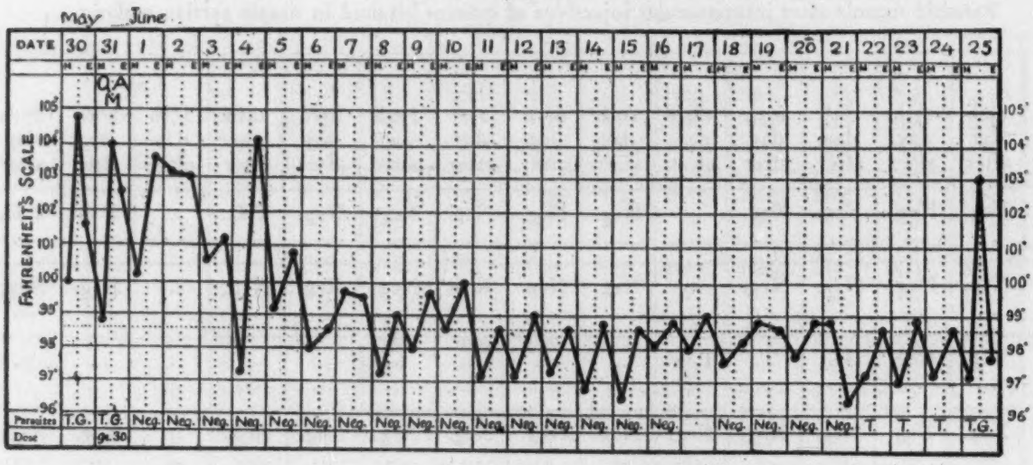
Number of Case		2nd day before	1st day before	Day of first injection	1st day after	2nd day after	3rd day after	4th day after	5th day after	6th day after	7th day after	8th day after	9th day after	10th day after	11th day after
102	Parasites ...	...	T.G.	T.G.	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	...
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...
103	Parasites ...	...	T.G.	T.G.	T.G.	T.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	...
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...
104	Parasites ...	T.	T.G.	T.G.	G.	Neg.	Neg.	Neg.	...	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...
105	Parasites ...	...	T.	T.G.	T.G.	G.	Neg.	Neg.	Neg.	Neg.	...	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...
106	Parasites ...	...	T.G.	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	...	Neg.
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...
107	Parasites ...	...	...	...	T.G.	Neg.	...	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	...	Neg.
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...
108	Parasites ...	T.	T.G.	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	...	Neg.	Neg.
	Dose in grains ...	...	...	15	15	...	...	...	...	...	...	...	...	...	...
109	Parasites ...	T.	T.G.	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	20	20	...	...	...	...	...	...	...	...	...	...
110	Parasites ...	T.G.	T.G.	T.G.	Neg.	Neg.	Neg.	...	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	20	20	...	...	...	...	...	...	...	...	...	...
111	Parasites ...	Neg.	T.G.	T.G.	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	20	20	...	...	...	...	...	...	...	...	...	...
112	Parasites ...	...	T.	T.	G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	30	30	...	...	...	...	...	...	...	...	...	...
113	Parasites ...	...	T.G.	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	30	30	...	...	...	...	...	...	...	...	...	...
114	Parasites ...	...	T.G.	T.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	30	30	...	...	...	...	...	...	...	...	...	...
115	Parasites ...	...	T.G.(Cr.)	T.G.	T.G.	(Cr.)	Neg.	Neg.	(Cr.)	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	30	30	...	...	...	...	...	...	...	...	...	...
116	Parasites ...	T.	T.G.	T.G.	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	30	30	...	...	...	...	...	...	...	...	...	...
117	Parasites ...	...	G.	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	30	30	...	...	...	...	...	...	...	...	...	...
118	Parasites ...	...	T.G.	T.G.	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	30	30	...	...	...	...	...	...	...	...	...	...
119	Parasites ...	...	T.G.	T.G.	T.G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	30	30	...	...	...	...	...	...	...	...	...	...
120	Parasites ...	...	T.G.	T.G.	T.G.	Neg.	G.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
	Dose in grains ...	...	...	30	30	...	...	...	...	...	...	...	...	...	...

TABLE II.—*continued.*

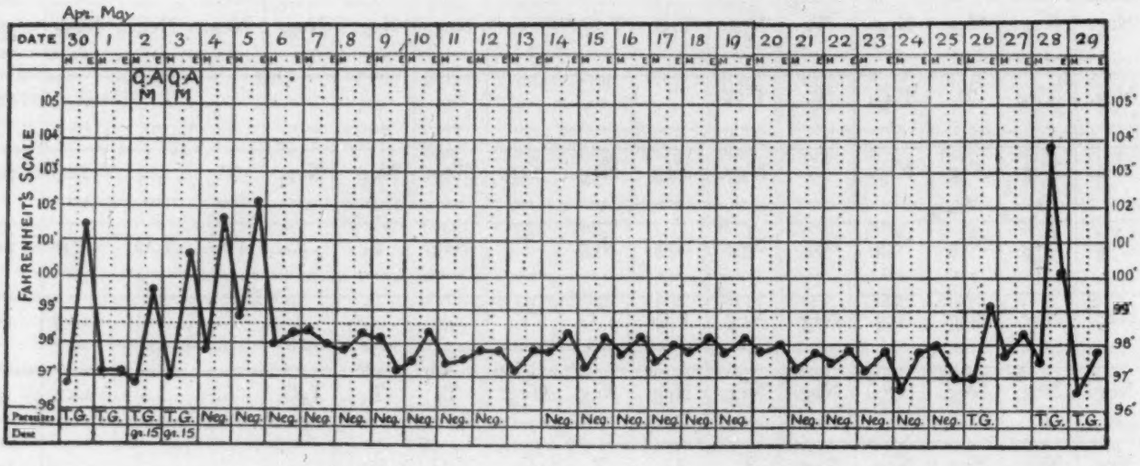
Parasitic records after intramuscular injections of quinine alkaloid in simple tertian malaria.

12th day after	13th day after	14th day after	15th day after	16th day after	17th day after	18th day after	19th day after	20th day after	21st day after	22nd day after	23rd day after	24th day after	25th day after	26th day after	27th day after	Number of Case
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	... ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	T.G. ...	... ...	T.G. ...	T.G. ...	102
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	... ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	... ...	Neg. ...	Neg. ...	103
... ...	Neg. ...	Neg. ...	Neg. ...	G. ...	T. ...	T.G. ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	104
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	... ...	Neg. ...	Neg. ...	105
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	106
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	T. ...	Neg. ...	T.G. ...	T.G.* ...	T.G.* ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	107
Neg. ...	Neg. ...	(Cr.) ...	T. ...	T.G. ...	T. ...	T.G. ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	108
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	... ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	T. ...	... ...	... ...	109
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	T. ...	Neg. ...	T. ...	T.G. ...	T.G. ...	T.G. ...	T. ...	110
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	T.G. ...	T.G. ...	T. ...	... ...	... ...	... ...	... ...	... ...	... ...	111
(Cr.) ...	Neg. ...	Neg. ...	Neg. ...	(Cr.) ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	T. ...	T. ...	T.G. ...	... ...	... ...	... ...	112
... ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	T. ...	G. ...	T. ...	G. ...	T.G. ...	G. ...	... ...	... ...	... ...	... ...	113
... ...	Neg. ...	Neg. ...	Neg. ...	T. ...	G. ...	T.G. ...	G. ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	114
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	T.G. ...	T.G. ...	T.G.* ...	T.G. ...	T.G. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	... ...	115
Neg. ...	Neg. ...	T. ...	T. ...	T.G. ...	... ...	T.G. ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	116
... ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg ...	Neg. ...	Neg. ...	T. ...	Neg. ...	T.G. ...	G. ...	... ...	... ...	... ...	... ...	117
Neg. ...	Neg. ...	Neg. ...	T.G. ...	T.G.* ...	T.G. ...	Neg. ...	Neg. ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	118
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	119
Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	... ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	Neg. ...	... ...	120

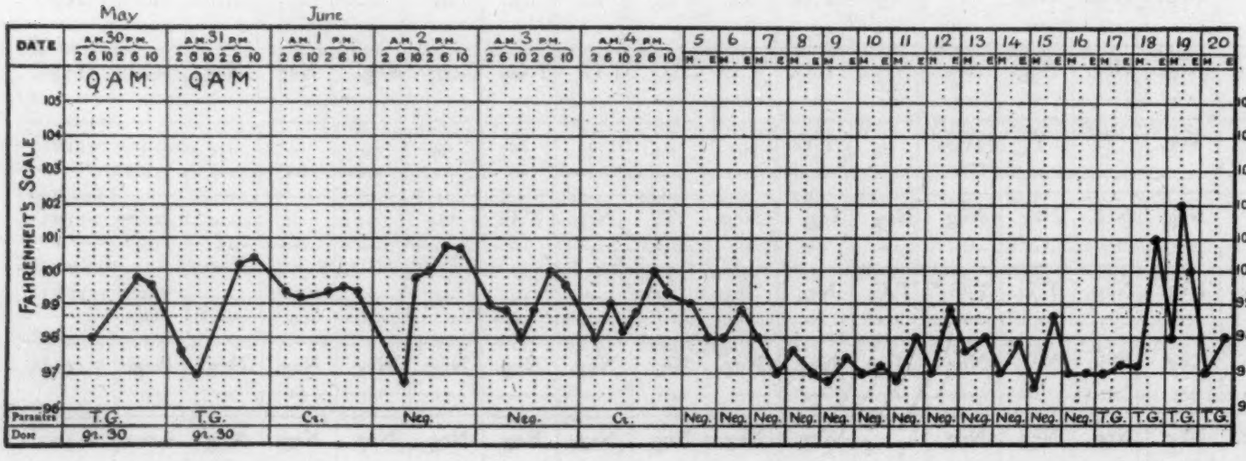
Case 87



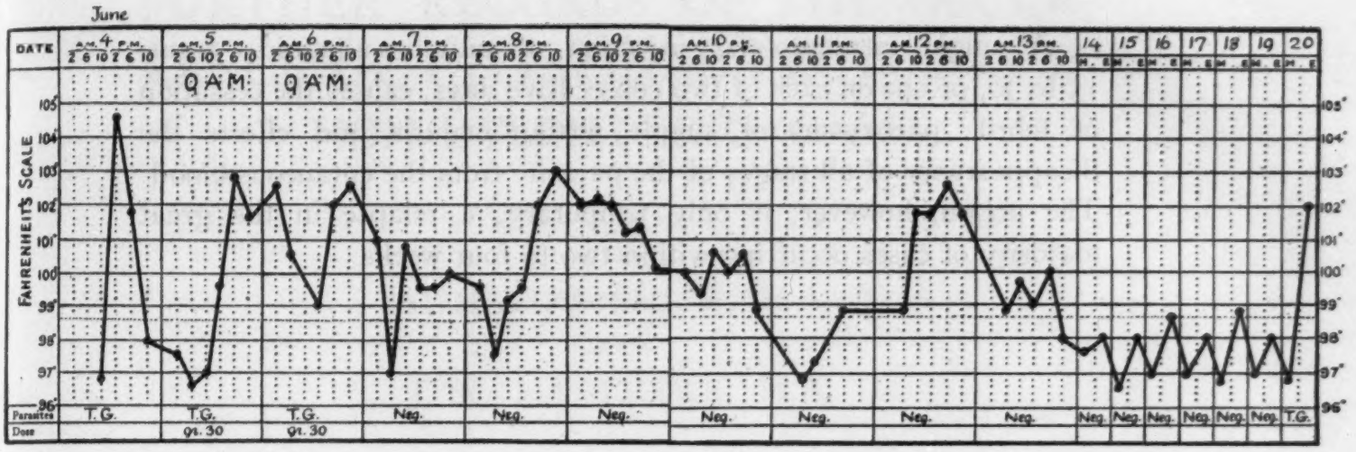
Case 102



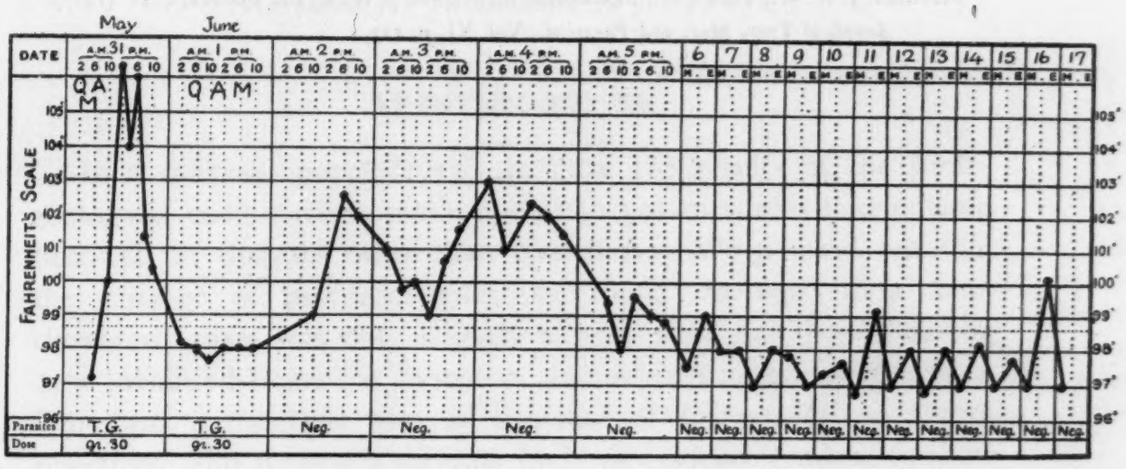
Case 115



CASE 118



CASE 119



EFFECTS OF THE INJECTIONS

Subcutaneous injections of this solution resulted in sloughing. Intramuscular injections of 3 to 4 c.c. resulted in slight pain at the time of injection; in larger doses, 6 c.c., the immediate pain was more severe and pain recurred some 24 to 48 hours later. There was no abscess formation or other ill effect. For several days after injection a slight diffuse swelling was appreciable at the site of injection; this, however, disappeared entirely in the course of a few days.

CONCLUSION

A single intramuscular injection of quinine alkaloid, grains 15 to 30, or one on each of two consecutive days, causes the cessation of febrile paroxysms of simple tertian malaria and effects the disappearance of all stages of the parasite from the cutaneous blood. The action, however, is only temporary, a relapse in the great majority of cases occurring within two to four weeks.

REFERENCE

STEPHENS, J. W. W., YORKE, W., BLACKLOCK, B., MACFIE, J. W. S., and COOPER, C. F. (1917). *Annals of Trop. Med. and Parasitol.*, Vol. XI, p. 113.

FURTHER RECORDS OF THE OCCURRENCE OF INTESTINAL PROTOZOA IN NON-DYSENTERIC CASES

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I. INTRODUCTION

In a previous paper (1917) we gave the results of the protozoological examinations made upon 250 patients who had entered hospital for diseases other than dysentery. We pointed out that the number of protozoal infections found amongst these non-dysenteric cases was only slightly smaller than that found among men invalided home for dysentery and related intestinal disorders. This was particularly true in the case of *Entamoeba histolytica* infections, and there is a growing body of evidence tending to show that this parasite is probably equally common among all classes of military patients in the present war, especially if they have come from an area where *E. histolytica* is endemic.

The results with which this paper deals have been obtained from the examination of 200 soldiers who were in hospital for various diseases, including malaria, pneumonia, pleurisy, hernia, haemorrhoids, rheumatism, wounds, etc. None of the cases was in hospital for dysentery. We endeavoured to examine a fairly large number of wounded men, but for various reasons we did not get the number we desired, and in order to obtain 200 cases altogether we had to include a large number suffering from malaria. On the whole, however, the present series is very similar in composition to our first

series of 250, but it does not include any patients who have never been out of England. Samples of the stools were collected in the morning, and the examinations were always conducted the same day. At least two preparations from each stool were carefully searched. The protozoal findings were as follows:

II. GENERAL RESULTS

Total number of cases examined 200
Number of cases having protozoal infections... 69
= 34·5 per cent.

An analysis of the 69 positive cases is given in the following table. For the purposes of comparison we have also given our previous results obtained from the examination of 250 non-dysenteric cases.

TABLE I.

	Number of cases infected	Percentage of all cases	Pure infections	Mixed infections	Our previous results from 250 cases
Entamoeba histolytica	15	7·5	4	11	8·0 %
Entamoeba coli	47	23·5	32	15	19·2 %
Giardia (= *Lamblia*) *intestinalis* ...	23	11·5	13	10	8·0 %
Chilomastix (= *Tetramitus*) *mesnili*	4	2·0	3	1	2·0 %
Trichomonas intestinalis	—	—	—	—	1·7 %

In numerous instances multiple infections occurred, and the details regarding these are as follows:

Double Infections:

E. histolytica and *E. coli* in six cases.
E. histolytica and *G. intestinalis* in two cases.
E. coli and *G. intestinalis* in six cases.

Triple Infections:

E. histolytica, *E. coli* and *G. intestinalis* in two cases.
E. histolytica, *E. coli* and *C. mesnili* in one case.

III. NUMBER OF EXAMINATIONS AND REAL INCIDENCE OF INFECTION

Before proceeding to discuss the general results presented in Table I, it is desirable to make reference to the number of examinations which were performed and by which the results were obtained. Upon our 200 cases 628 examinations were made, which is an average of about three examinations per case. Only 68 cases, however, actually received three examinations each, and only 24 had as many as six examinations. There is now abundant evidence to show that one or two examinations are quite inadequate to detect the majority of cases harbouring protozoal infections. Dobell (1917) has emphasised the inadequacy of even three examinations per case, and states that such a system would lead to the detection of no more than one-half to two-thirds of the cases infected with *E. histolytica*. Wenyon and O'Connor (1917) have shown that of twelve infections of *E. histolytica* ultimately found amongst ninety-two cases, only four were detected at the first examination, and argue that in order to obtain an approximately correct result the figures obtained from a single examination should be multiplied by three.*

In the Second Report (1917) on the examination of dysenteric cases issued from the Liverpool School of Tropical Medicine there is given, in Table XI, page 54, a series of figures relating to *E. histolytica* infections which indicate the value of the examinations from the first to the sixth. It is there shown that at the first examination there are detected only one-third of the infections to be found by six examinations per case, and only one-fourth of the total number of cases actually harbouring the infection. With these considerations before us we have no doubt that the figures given in Table I do not represent the real incidence of infection of the various protozoa recorded. They are the findings which have been obtained in the great majority of cases from one or two examinations only. The following table gives for the first to the sixth examination (*a*) the actual number of cases examined at each stage, (*b*) the findings of *E. histolytica*, (*c*) of *E. coli*, and (*d*) of *G. intestinalis*.

* In the table given by Wenyon and O'Connor, we can only find three *E. histolytica* infections detected at the first examination, which would give four as the factor for multiplication. Obviously, however, the number of cases is too small from which to obtain any reliable factor.

TABLE II

Examination	Number of cases examined (*a*)	Cases infected with *E. histolytica* (*b*)	Percentage of total cases examined	Cases infected with *E. coli* (*c*)	Percentage of total cases examined	Cases infected with *G. intestinalis* (*d*)	Percentage of total cases examined
First	200	12	6·0	32	16·0	13	6·5
Second	123	12	6·0	38	19·0	18	9·0
Third	68	14	7·0	43	21·5	20	10·0
Fourth	35	14	7·0	46	23·0	21	10·5
Fifth	27	14	7·0	47	23·5	21	10·5
Sixth	24	15	7·5	47	23·5	21	10·5
Ultimately		15	7·5	47	23·5	23	11·5

We will not enter into a full discussion of the figures presented in Table II, but it is worthy of note that in the small number of cases examined a third time two new *E. histolytica* infections were discovered, and among the twenty-four cases that were before us at the sixth examination one *E. histolytica* infection was found that had hitherto escaped detection. It will be noticed that the first examination resulted in the discovery of twelve cases (6 per cent.) positive to *E. histolytica*. In a much larger series of dysenteric cases (1,713) examined in this laboratory, 5·9 per cent. were found at the first examination to be infected with this parasite. It has been estimated that the real incidence of infection among this series of dysenterics was at least 20 per cent., and from the comparative figures we have just given, there seems every reason to believe that the incidence of infection with *E. histolytica* among our 200 non-dysenterics was equally high. The same remarks are true also for infections with *E. coli*. At the first examination we found 16 per cent. of the cases to be infected with this entamoeba. This figure is almost the same as that obtained at the corresponding examination in dysenteric cases, namely, 15·5 per cent. (see Table X, page 52, of Second Report), and it was estimated that at least 50 per cent. of these cases were really infected with *E. coli*. It

seems probable, therefore, that the real incidence of infection with *E. coli* amongst our non-dysenteric cases was also about 50%. In the column (*d*) Table II we give the findings of *G. intestinalis*, and it will be observed that two infections were found after the sixth examination. One of these was detected at the eighth and the other at the tenth examination. So far as we can judge from this, as well as from our first series of non-dysentericɜ, there appears to be a somewhat smaller number of cases infected with this flagellate than among dysenteric cases. A single examination of 1,713 dysentery cases proved 11·3 per cent. to be infected with *G. intestinalis*. Our non-dysenteric cases, however, give only 6·5% of infection at the first examination. We do not know if there is any significance in the difference. From the foregoing considerations, then, it seems probable that the incidence of infection with *E. histolytica* and *E. coli* is approximately the same in dysenteric and non-dysenteric cases, while the number of *G. intestinalis* infections appears only slightly smaller in the latter class of patient.

IV. DISCUSSION OF GENERAL RESULTS

It will be seen from Table I that our present findings are in close agreement with those recorded in our previous paper. It is noteworthy, however, that no infection with *Trichomonas* was discovered in our 200 cases. It is unnecessary for us to enter into a full comparison with the results of other workers. We made reference to some of these in our first paper, and Dobell (1917) in his Report to the Medical Research Committee, pp. 59-60, has given a summary of the findings of *E. histolytica* at various centres in this country among non-dysenteric patients. It is sufficient to remark that all the results point to the fact that a considerable percentage of *E. histolytica* carriers exists among men who have been invalided to this country, from regions where the parasite is endemic, for reasons quite unconnected with any attacks of amoebic dysentery. It is not surprising, therefore, to learn that among healthy troops and natives in Egypt there occurs a similarly large percentage of infected individuals. Wenyon and O'Connor (1917) have recently published abundant evidence of this, and we will refer briefly to only a few of their results. Among 1,979 healthy troops these

authors found 5·3 per cent. infected with *E. histolytica*, a result which is quite comparable with our own, for it is based on a single examination. Among 524 healthy natives 13·7 per cent. were found to be harbouring *E. histolytica*, and thus the source of infection was clearly indicated. Ravaut and Krolunitski (1916) found cysts of *E. histolytica* in 5 per cent. of apparently healthy individuals in one district in France.

V. SPECIAL ASPECTS OF THE RESULTS

In order to make a fuller comparison between the results obtained from the present series of cases and our first series, we shall follow the method we previously adopted and arrange our cases in certain categories.

A. THE RELATION OF THE INFECTIONS TO THE DISEASES SUFFERED BY THE PATIENTS

As only 15 of our 200 cases had entered hospital for intestinal complaints we cannot satisfactorily compare the number of infections among these with the number found among the remaining 185 non-intestinal cases. We wish, however, to refer to a particular class of patient—those who had been invalided home with malaria. We examined 100 patients suffering from this disease. Fifty-three of these received only a single examination, yet we found among the hundred cases six infections with *E. histolytica*, five of these being discovered at the first examination; only one of these six cases had a history of dysentery. If one hundred cases may be taken as a fair sample of the large numbers of men invalided with malaria, it follows from our findings that considerable numbers of soldiers suffering from malaria are acting as carriers of *E. histolytica*.

B. DISTRIBUTION OF THE INFECTIONS BETWEEN PATIENTS WITH PREVIOUS HISTORY OF DYSENTERY OR DIARRHOEA AND PATIENTS WITH NO SUCH HISTORY

We have already mentioned that none of the patients were in hospital for dysentery, yet some of them had suffered from this disease previous to the date of their return to this country. The

information on this point was obtained by questioning each patient, and dysentery has been recorded only in those instances where all the evidence suggests that the patient actually had suffered. It has been impossible, however, to discover whether the past illness was due to bacillary or amoebic infection.

In Table III we give the details of the infections found in the two categories of cases.

TABLE III.

History of Dysentery		No history of Dysentery	
Number of cases examined ... 42		Number of cases examined ... 158	
	Number of cases infected		Number of cases infected
E. histolytica	3 = 7·1 %	*E. histolytica*	12 = 7·6 %
E. coli	11 = 26·2 %	*E. coli*	36 = 22·8 %
G. intestinalis	1 = 2·4 %	*G. intestinalis*	22 = 13·9 %
C. mesnili	1 = 2·4 %	*C. mesnili*	3 = 1·9 %

Since the number of patients with a history of dysentery is small, it would be unsafe to come to definite conclusions from the two sets of figures given in the above table. It is worthy of remark, however, that in each category there is very little difference in the percentage of amoebic infection, and we may recall that in our previous paper we recorded 7·7 per cent. of infection with *E. histolytica* among 220 patients with no previous history of dysentery. This figure is almost exactly the same as given above for the 158 cases which fall in a strictly similar category. It seems probable that there is little difference between the percentage of *E. histolytica* carriers among men who have had dysentery and the percentage of men who become carriers without suffering from dysentery at all.

We may here mention some of the results of Wenyon and O'Connor, who examined a large series of cases in Egypt. Of 1,383 healthy men who had served in both Gallipoli and Egypt, 246

had a history of dysentery, and of these, 6·5 per cent. were infected with *E. histolytica*. Among the remaining 1,137 who had no history of dysentery 4·5 per cent. were infected. The percentages of *G. intestinalis* infection which are recorded in Table III are rather surprising, although of course we cannot lay too much stress on the result obtained from so small a number as 42. The records, however, are of some importance in considering whether *G. intestinalis* is a causal organism of dysentery.

In order to make further comparisons among our cases we ascertained which patients had a previous history of diarrhoea, and in Table IV we compare the findings amongst those with a history of dysentery or diarrhoea with those who have had neither of these ailments.

TABLE IV.

History of Dysentery or Diarrhoea		No history of Dysentery or Diarrhoea	
Number of cases examined ... 98		Number of cases examined ... 102	
	Number of cases infected		Number of cases infected
E. histolytica	9 = 9·2 %	*E. histolytica*	6 = 5·9 %
E. coli	26 = 26·5 %	*E. coli*	21 = 20·6 %
G. intestinalis	10 = 10·2 %	*G. intestinalis*	13 = 12·7 %
C. mesnili	4 = 4·1 %	*C. mesnili*	0

We have in each category about an equal number of cases, and the number is sufficiently large to give fairly reliable results. The amoebic infections are somewhat more prevalent among patients who have had dysentery or diarrhoea, but it is nevertheless true that a considerable number of infections occur among patients who have no history of either. The finding of so many infections of *G. intestinalis* among patients who have never had dysentery or diarrhoea is particularly noteworthy, and in conjunction with the record given in Table III, is a fact of some importance in any discussion regarding the pathogenicity of this flagellate.

C. CLASSIFICATION OF PATIENTS ACCORDING TO THE REGIONS IN WHICH THEY HAVE TRAVELLED

In our former contribution we dealt with four categories under this heading. In the present series we deal with only two (1) men who have resided or travelled in tropical or sub-tropical regions, (2) men who have been to France only. The results obtained from the examinations of persons who have never been out of Great Britain have been given in another paper (1917). Table V deals with our present series of 200, and shows the distribution of the various infections in the two categories.

TABLE V.

Resided or travelled in tropical or subtropical regions		Resided in France and England only	
Number of cases examined ... 142		Number of cases examined ... 58	
	Number of cases infected		Number of cases infected
E. histolytica	11 = 7·7 %	*E. histolytica*	4 = 6·9 %
E. coli	33 = 23·2 %	*E. coli*	14 = 24·1 %
G. intestinalis	16 = 11·2 %	*G. intestinalis*	7 = 12·1 %
C. mesnili	4 = 2·8 %	*C. mesnili*	0

We wish we could have examined a larger number of cases from France, but the striking fact about the percentages given in the two columns of Table V is their close agreement, at least for *E. histolytica*, *E. coli* and *G. intestinalis*. The results for the two latter protozoa support our previous findings among 91 men from France, 23·1 per cent. of whom were infected with *E. coli* and 8·4 per cent. with *G. intestinalis*. We found, however, only two cases (2·4 per cent.) infected with *E. histolytica*. That we should have discovered in our present small series of 58 cases from France as many as four *E. histolytica* infections is, we think, a fact of considerable significance. These four men before the war had never

been out of England, and two of them had each spent less than six months in France. The other two had each spent a longer time, and both had records of 'severe diarrhoea' lasting about a fortnight with ten to fifteen stools a day. Presumably men who go from this country to France are exposed to infection with *E. histolytica* through coming in contact with 'carriers' returned from regions where the parasite is most prevalent. That this exposure does not exist without a certain risk is indicated by the record of the two cases we have mentioned above.

Of the total fifteen *E. histolytica* cases found we wish to record that in twelve instances the cysts were all of the more usual size measuring on the average about 12μ in diameter; in two cases the infection consisted entirely of the smaller form of cysts measuring 7μ to 8μ, while in one instance there occurred both large and small cysts. One of the cases having small cysts only was from France. Detailed observations on these small cysts of *E. histolytica* have recently been published by Wenyon and O'Connor (1917), and Dobell and Jepps (1917).

Finally we may record the occurrence of the eggs of *Trichocephalus dispar* in five cases and *Ancylostoma duodenale* in two cases. In seven instances an infection of 'I bodies' was observed.

We would again express our indebtedness to Dr. Abram for allowing us to work in the wards of the Royal Infirmary under his charge, and to the sisters and nurses of these wards we are grateful for their attention in the supply of material. We wish also to thank Dr. D. L. Mackinnon and Mr. H. F. Carter for their help in making the microscopic examinations.

SUMMARY

1. Of 200 non-dysenteric patients in hospital for various diseases 34·5 per cent. were found to be infected with various protozoa. *E. histolytica* was discovered in 15 cases (7·5 per cent.).

2. One hundred cases were malaria, and among these six infections with *E. histolytica* were detected.

3. There were 42 cases with a history of dysentery and 158 cases with no history of dysentery. Of the former 7·1 per cent.

were found positive to *E. histolytica*, and of the latter 7·6 per cent.

4. Of 142 patients who had been to tropical or sub-tropical regions 11, or 7·7 per cent., were found infected with *E. histolytica*. The remaining 58 cases had been to France only, and four, or 6·9 per cent., were discovered to be infected with this parasite.

5. The great majority of the cases (123) had only two examinations each.

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THE VALUE OF CONCENTRATING THE CYSTS OF PROTOZOAL PARASITES IN EXAMINING THE STOOLS OF DYSENTERIC PATIENTS FOR PATHOGENIC ENTAMOEBAE

BY

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From the Liverpool School of Tropical Medicine

(*Received for publication* 17 *July*, 1917)

A method of concentrating Entamoeba cysts in stools has recently been described by Cropper and Row (1917). Details of two processes are given by these authors: (1) a maximum concentration of protozoal cysts for purposes of diagnosis; (2) a relative concentration for purposes of cultivation. The authors claim that the cysts are much more easily found and more easily identified in faecal material treated by their first method than in preparations made in the ordinary way from untreated material. In addition they maintain that the errors which arise from the examination of a very small sample of untreated material are almost wholly eliminated when the concentration method is employed. With a view to forming some idea of the extent of these errors in regard to the findings of *Entamoeba histolytica*, *E. coli* and *Giardia intestinalis* the concentration method was adopted in the examina-

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tion of a number of stools that had previously been examined in the usual manner. In the great majority of the cases thus dealt with, the ordinary examinations performed had given negative results. Post-treatment observations were also made on a number of *E. histolytica* cases in order to discover the possibility of cysts being passed in such small numbers that they might escape detection by ordinary methods of examination. Finally, a few special cases known to be infected with particular protozoa were examined almost daily by both methods so that the separate records of results obtained could be compared in detail.

In all our experiments we have followed fairly closely the technique employed by Cropper and Row for obtaining a maximum concentration of cysts from the portions of stools sent for examination. They recommend that at least 1 gram of faecal material be shaken up with about 30 c.c. of normal saline for half an hour. Unfortunately, owing to the difficulty of obtaining moderately large samples of faeces, we have frequently been obliged to make use of material weighing less than the minimum suggested. This fact may have appreciably affected the results obtained, as experiments made with varying amounts of material show that with less than 1 gram the concentration is of less value than when 2 or 3 grams are used. After the necessary shaking with salt solution has been completed the faecal emulsion is shaken up with about one-eighth its volume of ether, and the mixture transferred to a separating funnel and allowed to stand for a short time. During this period the liquids separate and the faecal débris which has absorbed ether is carried to the surface. The cysts remain in the saline solution below the ether layer and gradually sink to the bottom of the separating funnel. Cropper and Row allowed the mixture to stand for a minute or two and then drew off the saline into 15 c.c. centrifuge tubes. We used centrifuge tubes of 10 c.c. capacity, but in order that the liquid first drawn off might contain a greater proportion of cysts we allowed the mixture to remain in the funnel for five minutes or more. The length of time allotted to this stage of the process seems to us of some importance, particularly in cases where small cysts are suspected. The smaller and lighter cysts of *E. histolytica* and *G. intestinalis* do not fall to the bottom of the funnel so quickly as do those of *E. coli*. If the emulsion has been allowed to stand in

the funnel for five minutes or longer, the first sample which is drawn off into the centrifuge tube has been found to give a much higher concentration value than any subsequent sample. After centrifugalisation the supernatant liquid is decanted, and a preparation is made from a loopful of the sediment. If the sediment brought down was very considerable, fractional centrifugalisation with salt solution was resorted to and the coarser particles removed. Our general practice when centrifuging was to give three minutes at a speed of 1,400 revolutions per minute. This thorough centrifugalisation was always given, since we were working to a large extent with material which had been declared negative by ordinary methods and in which cysts, if present, would be few in number.

In conducting ordinary examinations on fresh material our rule has been to make two preparations in saline of the material submitted. Throughout this article we shall refer to examinations of such preparations as *ordinary examinations*, and to those made on the sediment obtained by the process described by Cropper and Row for concentrating the cysts as *concentration examinations*.

We have examined in all 178 cases, upon which 523 concentration examinations have been performed. Of these 178 cases, 45 were either infected with *E. histolytica* or were specially selected cases kept under frequent observation both by ordinary and concentration methods of examination. Upon these 45 cases, 390 concentration examinations were made, and remarks on the results obtained will be given later. The remaining 133 cases were all negative to *E. histolytica* in each of three ordinary examinations made at distinct intervals. Each case was examined by the concentration method once only, the examination generally being performed immediately after the third ordinary examination had been made, and always upon material from the same stool upon which this test had been conducted. Although the above 133 cases were recorded negative to *E. histolytica* they included a few cases having infections of *E. coli* or *G. intestinalis*. The inclusion of these, however, does not interfere with our results, since our primary object was to ascertain if any *E. histolytica* infections were escaping detection in the ordinary routine method of examining the stools.

Our findings among the 133 cases examined are presented in the following table:—

TABLE I.

	Number of cases found infected after three ordinary examinations	Number of cases found infected after one concentration examination	Additional infections discovered by the concentration examination
E. histolytica	0	5	5
E. coli	11	23	12
G. intestinalis	12	16	4

Since the cases dealt with in the above table received only one concentration examination each, it is almost certain that the number of positive findings is lower than it would have been had each received more than one. It is now generally recognised that a large number of ordinary examinations (in our own experience we have made as many as 45) are occasionally necessary to detect an infection, and it is shown later (page 203) that in a lesser degree this applies to concentration examinations also. The performance of a single concentration examination giving a negative result does not necessarily mean that the stool is uninfected, and we have evidence to show that several examinations of this kind are required before it could safely be concluded that a particular case harboured no intestinal protozoa. The results we have obtained, however, show that a single examination by the concentration method will frequently result in the discovery of cysts of protozoal parasites when three ordinary examinations have failed to detect their presence. Our results seem to be due to the fact that an infected patient may pass a very small number of cysts in his stool, and that only by concentration is there a reasonable chance of discovering them. Furthermore the continued observations which have been made on selected cases have led us to consider the possibility of a patient, known to be infected, passing stools containing no cysts at all. Such cases, where cysts are rare in the faeces, are obviously likely to be returned as negative unless prolonged and tedious searching of the material submitted be made or unless continued observations on the case are possible. It is under such conditions—

after the first two or three ordinary examinations have proved negative—that the chief value of a single examination by the concentration method becomes apparent.

Among the 133 cases examined by concentration, five *E. histolytica* infections were found in spite of the fact that these cases had been subjected to the fairly severe test of three ordinary examinations. At the third examination, therefore, 3·8 per cent. of the cases recorded negative to *E. histolytica* were actually positive. Taking the percentage of cases infected with *E. histolytica*, after three ordinary examinations have been made upon their dejecta, as 11·5, a single concentration of the negatives might be expected to raise the incidence of infection to 14·9 per cent. The latter percentage would undoubtedly have been further increased had the negatives been concentrated at *each* of the ordinary examinations, and indeed the concentration method might be employed to obtain, in a practical manner, an approximation to the real incidence of infection.

By means of the three ordinary examinations *E. coli* was shown to be present in 11 of the 133 cases, so that the 12 additional infections with this entamoeba found by the concentration examination were derived from the 122 remaining cases. Thus about 10 per cent. of the cases apparently negative to *E. coli* actually harboured the infection. Our experience in examining dysenteric cases is that about 30 per cent. are found infected with *E. coli* after three examinations. By a single concentration this figure would be raised to about 37 per cent.

As shown in the table, four infections of *G. intestinalis* were discovered by concentration; 12 of the total cases examined had been previously determined positive to this organism and 121 negative. Of these latter cases, however, at least 3·3 per cent. were really positive, and it follows therefore that the figure, say 18·5 per cent., which may be taken to represent the incidence of infection with this flagellate after three ordinary examinations would be raised to about 21 per cent. as a result of one concentration examination per case of the negative cases.

Some statement is perhaps necessary regarding the figures (11·5, 30 and 18·5) which have been chosen as indicating the percentages of *E. histolytica*, *E. coli* and *G. intestinalis* cases

to be found by three ordinary examinations.* These are derived from the statistical considerations presented in the Second Report on the examination of dysenteric cases at the Liverpool School of Tropical Medicine (1917). It is also of some interest to compare our results with the calculations given in Tables IX, X, and XI of that report. From such a comparison it appears that the findings obtained by a single concentration examination, made at the time of the third ordinary examination, would raise the incidence of infection in the cases of *E. coli* and *G. intestinalis* to a figure as high as that which should be obtained by five ordinary examinations per case. The figure which would be reached in the case of *E. histolytica* is lower than that which should be obtained by five ordinary examinations, but is higher than that obtained by four.†

Although not considered of primary importance in this investigation, a few remarks on the records relating to the detection of helminthic ova in the stools examined may here be made. Of the total 178 cases upon each of which one or more concentration examinations were performed, six cases were found infected with *Trichiuris trichiura* (= *Trichocephalus dispar*) by the first three ordinary examinations performed. A concentration made at this stage, however, showed that the eggs of this worm were present in the stools of 19 (11 per cent.) of the 172 remaining cases. Of greater interest was the discovery in one case of the eggs of the pathogenic species *Ancylostoma duodenale*, and in another case the eggs of *Taenia saginata*. By ordinary methods and a limit of three examinations per case, these infections would not have been detected.

Among the 45 cases mentioned on page 197 were 35 patients who had been positive to *E. histolytica* and who had undergone treatment with emetine bismuth iodide or with alcresta ipecac. These cases were examined daily by the ordinary method with a view to detecting relapse and in order to ascertain whether any cysts were

* In this connection it must be remembered that the 133 cases selected for concentration do not form an average sample of the large series of cases examined at this laboratory. We endeavoured, in fact, to examine as many apparently negative cases as possible and therefore the numbers of cases (11 and 12) recorded positive to *E. coli* and *G. intestinalis* by the ordinary method are necessarily small.

† In Tables IX, X, and XI referred to above it is calculated from observations made upon 1,713 cases, that by five examinations per case, *G. intestinalis* should be found in 21·4 per cent., *E. coli* in 37·1 per cent., and *E. histolytica* in 15·9 per cent.

escaping detection, despite the severity of the tests afforded by prolonged observation, a single concentration examination was made on the stool of each patient shortly before his discharge from hospital took place. This examination was performed on the average at the twenty-fourth day after treatment had ceased. No relapses were found among the cases thus examined.

We have previously stated that a small number of selected cases were followed almost daily both by ordinary and concentration methods of examination. This was done with the object of obtaining a series of records which might give some indication of the comparative value of the two methods. In all, 16 cases came within this category, and with few exceptions were kept under continued observation for from five to seven weeks. In Table II there is presented the record of a case having an infection of *E. histolytica* and *E. coli* which may be taken to illustrate certain points of interest and importance.

Samples of the stools of this patient were sent to the laboratory for examination in the ordinary way, and on March 7th the third test was made. This, as well as the first two examinations, was recorded negative, and under ordinary circumstances the patient would now have been discharged as uninfected. On the date of the third examination, however, a concentration examination was performed, and an extremely scanty infection of *E. histolytica* and *E. coli* was detected. Thereafter frequent observations were made, and, as will be seen from the record, it was not until the eleventh ordinary examination that the *E. histolytica* infection was discovered, and the seventh before *E. coli* was found. The significance of these observations lies in the fact that so many ordinary tests were performed before the infections were discovered. The infections were, in this particular instance, exceedingly scanty, but we have no reason to believe that in this respect we happened to be dealing with an exceptional case. Indeed, we are rather inclined to think that in any large series of cases there must be numerous instances similar to the one we have just described, and it is obvious that in such cases any protozoal infection is almost certain to escape detection when only a small number of ordinary examinations are made. Treatment with biniodide was administered between March 31st and April 9th, and post-treatment observations seem to indicate that the *E. histolytica* infection was removed. In regard

TABLE II.

DATE, 1917		Jan. 29	Feb 5	March 7	21	22	23	24	26	28	29	30	31	April 2	4	5	9	11	12	16	19	20	21	23	27	30	May 2	5
E. histolytica cysts	R*	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	...	–	–	–	–	–	–	–	–	–	–	–
	C	...	...	+	–	–	–	–	+	–	+	...	–	–	–	–	...	–	–	–	–	–	–	–	–	–	–	–
E. coli cysts	R.	–	–	–	–	–	–	+	+	+	+	–	–	–	–	–	...	–	–	–	–	–	–	+	+	+	+	+
	C	...	...	+	–	–	–	+	+	+	+	...	+	+	+	+	...	+	+	–	–	–	+	+	+	+	+	+

*R = Ordinary routine examination ; C = Concentration examination ; – = Negative ; + = Positive ; no entry = no examinations made. The heavy vertical lines indicate the beginning and end of treatment, the patient receiving a 10 days' course of emetine bismuth iodide.

to the *E. coli* infection, it will be noticed that, by the ordinary method of examination, no cysts of this organism were found in the stools during the treatment period and for some time afterwards. This negative interval might be attributed to the effect of the drug, but the concentration examinations showed that, for the greater part of the interval, cysts were regularly present in the faeces, although only in very small numbers. On the other hand, it must be observed that the disappearance of the organism, as determined by ordinary methods, did not exactly coincide with the administration of the drug, and the negative interval may only be a repetition of that which occurred before any treatment was given. Further, the record previous to the period of treatment shows in the case of *E. histolytica* five occasions, and in the case of *E. coli* three occasions, on which no cysts were found by concentration examinations. Whether, in this instance, the failure to discover cysts on these days indicated an entire absence of them in the stools, or whether the infection was so exceedingly scanty that even the concentration method did not succeed, we are unable to say. In this connection some remarks on another of the cases under constant observation infected with *E. coli* and *G. intestinalis* may be of interest. The *E. coli* infection was fairly heavy, and was discovered early by both methods of examination. The *G. intestinalis* infection was exceedingly scanty, and cysts were first found at the fifteenth concentration examination. No forms of this flagellate were ever detected in the stools by ordinary examinations, and only on three occasions were the cysts found by concentrating, although a total of 28 examinations were performed by each method.

The question as to whether a stool is absolutely or only relatively negative to protozoal organisms is one which cannot be determined by an ordinary routine test. For the concentration method of examination we are only prepared to say that the test is considerably more severe. In this respect it is of value, and might be conveniently adopted if it were urgently necessary to supply the result of a single protozoological examination. But when large numbers of stools have to be dealt with each day the method seems to us to be quite impracticable. The time required to examine a large series of cases even once by the concentration method is not commensurate with the results obtained. As we have previously indicated, a

concentration examination, performed at the third routine test, gives a result which would probably be obtained by five ordinary examinations.

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SOME RESULTS OBTAINED BY HEISER'S TREATMENT OF LEPROSY IN NIGERIA

BY

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PLATES IV-V

The method of treatment of leprosy, described by Heiser (1914), U.S. Public Health Service, has been tried with some of the inmates of the Yaba Leper Asylum.

The treatment was begun in May, 1916, so that it is as yet too soon to make a complete report, more particularly as all the patients are old-standing cases.

The outstanding results only are described, and some photographs are attached.

The oil-mixture used was that recommended by Heiser, Chaulmoogra oil 60 c.c., camphorated oil 60 c.c., and resorcin 4 grains. The mixture was sterilised by boiling, and 2 c.c., injected intramuscularly into the buttock, were given as an initial dose. After the lapse of one week, 3 c.c. were given, and this dose was increased by 1 c.c. per week until 8 c.c. were being administered. Beyond this amount some discomfort was complained of, so that the procedure finally adopted was to inject 6 c.c. twice a week. This dose was well tolerated and every case responded to treatment. The most rapid and obvious effect was the healing of ulcers, many of them large and deep, and of many years' duration. Softening, then absorption, of the nodules, fading of the maculae and the return of sensation were also observable, even to the patients themselves.

Seven cases have been selected for description.

CASE 1. Male. Age 38 years. Inmate of Asylum for four years. First noticed the disease eleven years ago.

Face leonine. Skin of face infiltrated and yellowish in colour. Nodules on cheeks, ears, lips and nose. Anaesthetic patches, both arms and both legs. Maculae right hand and both legs.

Treatment begun May 19th, 1916.

By December, 1916, the nodules had been considerably absorbed, particularly in the lips, nose and ears. The photograph shows distinct wrinkling where absorption has taken place, and the lips, nose and ears are smaller. (Plate IV, fig. 2.)

Sensation has returned in both arms, but only slightly in the legs, whilst the maculae have disappeared except from the lower part of the legs.

CASE 2. Male. Age 21 years. Inmate of Asylum for six years. First noticed the disease seven years ago.

Leonine expression. Skin of face thickened. Large nodules over and between eyebrows and on nose, cheeks, ears and lips. Anaesthetic patches, both arms and both legs. Maculae on shoulders, back, buttock, chest and abdomen.

Treatment begun May 19th, 1916.

By December, 1916, the nodules on cheeks, lips, ears and nose had in great part been absorbed. (Plate IV, fig. 4.) The anaesthesia had disappeared from the arms except for a small area on the left.

The maculae had completely disappeared.

CASE 3. Male. Age 40 years. Inmate of Asylum for 11 years. First noticed the disease 24 years ago.

Greater part of both arms from elbow downwards, anaesthetic. Similarly with both legs from knee downwards, and also an area above right knee. Also two areas on the back of the trunk. Many maculae on chest, abdomen, shoulders and back.

Treatment commenced May 19th, 1916.

By December sensation had returned to both arms except from the wrist downwards.

The anaesthesia had also disappeared from the area above the right knee, and below the knee in both legs a slight recovery of sensation had taken place.

The maculae had completely disappeared except from the abdomen, where they were becoming more faint.

Case 4. Male. Age 30 years. Inmate of the Asylum for three years. First noticed the disease seven years ago.

Both arms anaesthetic, left from two inches above elbow, and right from just below elbow, downwards. Patches of anaesthesia over left ankle and foot; right leg from below knee completely anaesthetic. A deep ulcer, active over two years at the base of left middle finger, extending down into the palm. An ulcer also on stump of left ring finger.

Treatment begun May 19th, 1916.

By December sensation had returned to the left arm as far as the elbow (below there was still anaesthesia) and in the right arm sensation was present as far as the wrist. There was complete sensation in the left leg, but only a slight return in the right.

Both ulcers had completely healed. (Plate V, fig. 6.)

Case 5. Male. Age 45 years. Inmate of Asylum for seven years. Disease started 'when a small boy.'

Anaesthesia over left arm from elbow downwards except for a small area at back of wrist. The left knee and foot anaesthetic. Patches of anaesthesia back and front right leg, below knee. A large deep ulcer 2½ inches in diameter on sole of left foot.

Treatment begun September 28th, 1916.

By December sensation had been completely restored to the left arm and right leg. Over the left knee also sensation had returned, but there was little improvement below the ankle.

The ulcer had completely healed. (Plate V, fig. 7.)

Case 6. Male. Age 25 years. Inmate of Asylum for two months. First noticed the disease two years ago.

The face and the back of the head and neck, with numerous maculae, all anaesthetic.

Treatment begun June 5th, 1916.

By December, 1916, sensation returned, except in a small patch over occipital prominence.

Maculae have disappeared except from left cheek.

Case 7. Female. Age 50 years. Inmate of Asylum for 12 years. Disease started 24 years ago.

Both wrists and hands anaesthetic, also some anaesthetic patches on right upper arm. Complete anaesthesia lower two-thirds of left leg. Maculae on left breast and both upper arms. A large deep, ragged ulcer middle third of left sole. Another ragged ulcer over area formerly occupied by base of left toes.

Treatment begun September 28th, 1916.

By December, 1916, sensation had been restored in the right

upper arm, and there was a slight improvement at the wrists. Sensation had returned half way down the left leg. The ulcer which occupied the area at the base of the toes had healed and cicatrised, and the ulcer on the sole was filling up with healthy granulations. (Plate V, fig. 9.)

Thanks are gratefully given to Dr. Pickels, Principal Medical Officer, Northern Provinces, Nigeria, for suggesting this course of treatment.

It should be added that Guaiacol carbonate, in 5 grain doses, twice daily, was found useful in controlling the febrile disturbance.

THE MEDICAL RESEARCH INSTITUTE,
LAGOS, *2nd February*, 1917.

REFERENCE

HEISER, VICTOR G. (1914). Leprosy: Its Treatment in the Philippine Islands by the Hypodermic Use of Chaulmoogra Oil Mixture. *Amer. Journ. Trop. Dis. & Prevent. Med.*, Vol. II, pp. 300-326.

EXPLANATION OF PLATES

PLATE IV

Fig. 1. Case I. May, 1916, before treatment.

Fig. 2. Case I. December, 1916, after treatment.

Fig. 3. Case II. May, 1916, before treatment.

Fig. 4. Case II. December, 1916, after treatment.



Fig. 1



Fig. 2



Fig. 3



Fig. 4

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Plate V

Fig. 5. Case IV. May, 1916, before treatment.

Fig. 6. Case IV. December, 1916, after treatment.

Fig. 7. Case V. December, 1916, after treatment.

Fig. 8. Case VII. September, 1916, before treatment.

Fig. 9. Case VII. December, 1916, after treatment.



Fig. 5

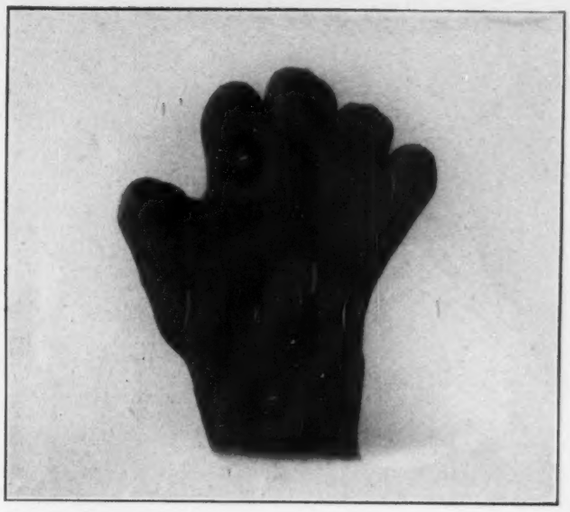


Fig. 6

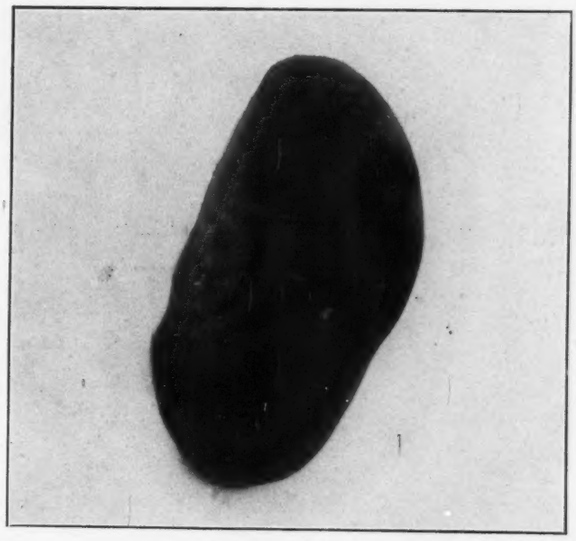


Fig. 7

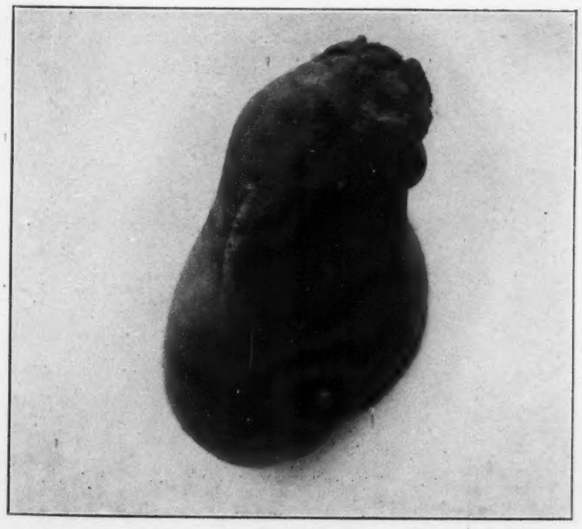


Fig. 8



Fig. 9

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CHILOMASTIX MESNILI
(WENYON, 1910)

BY

ALBERT J. CHALMERS, M.D., F.R.C.S., D.P.H.

DIRECTOR, WELLCOME TROPICAL RESEARCH LABORATORIES, KHARTOUM

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CONTENTS

		PAGE
I.	INTRODUCTORY	213
II.	HISTORY	214
III.	GEOGRAPHICAL DISTRIBUTION	222
IV.	ZOOLOGICAL DISTRIBUTION	222
V.	THE LIVING PARASITE	222
VI.	MORPHOLOGY	224
VII.	DEVELOPMENT	228
VIII.	CLASSIFICATION	231
IX.	TETRAMITIDAE	233
X.	GENERA	247
XI.	AFFINITIES	251
XII.	SUB-FAMILIES	251
XIII.	TETRAMITIDINAE	253
XIV.	GENUS CHILOMASTIX	254
XV.	CHILOMASTIX SPECIES	254
XVI.	CHILOMASTIX MESNILI	256
XVII.	PATHOGENICITY	257
XVIII.	TREATMENT	257
XIX.	SUMMARY	258
XX.	ACKNOWLEDGMENTS	258
	ADDENDUM	259
	REFERENCES	260
	EXPLANATION OF PLATES	262

I. INTRODUCTORY

In the course of enquiries into the diarrhoeas and dysenteries of the Anglo-Egyptian Sudan we have found that *Chilomastix mesnili* (Wenyon, 1910) is very frequently present in the motions of Europeans and Natives alike. We have never found it to be present entirely by itself, as it is, in our experience, always associated with one or more of the following organisms:—*Löschia coli*, *Löschia histolytica*, *Entamoeba nana* Wenyon and O'Connor 1917,

Blastocystis hominis, *Giardia intestinalis*, *Octomitus hominis*, or *Trichomonas intestinalis* synonym *T. confusa* Stiles, but we did not not observe it in the one case of infection with *Enteromonas hominis* da Fonseca 1915, which we have found here.

Although it seems quite harmless when present in small numbers, still we believe that when present in large numbers it may be the causal agent of diarrhoea, and, therefore, because it is such *a very common* intestinal flagellate it merits consideration.

Moreover, if only casually inspected in the living and moving condition in the faeces it is apt to be mistaken for a *Trichomonas*, and in its preserved and stained condition it has so frequently confused observers as to its morphology, even down to the present year, that we feel justified in attempting to clear up a few points. The following remarks are illustrated by photomicrographs, and though it must be admitted that it is impossible to obtain entirely satisfactory photographs of these minute animals, still photographs exclude the personal equation which must always be present in drawings.

II. HISTORY

The history of *Chilomastix mesnili* may be divided into three periods as follows:—

1. The Unrecognised Parasite.
2. Wenyon's Discovery.
3. Present Day.

1. *The Unrecognised Parasite.* The first record of this parasite, with which we are acquainted, is to be found in Davaine's '*Traité des Entozoaires*' published in 1860. On page 6 of the Synopsis with which this work begins he describes two varieties of *Cercomonas hominis*, viz., a large and a small. The large variety he obtained from the motions of cholera patients during the epidemic of 1853-1854 in Paris. So abundant were they that, at times, every drop examined contained many specimens. In this variety of *C. hominis* he saw with difficulty a flagellum arising from the broad end of the pear-shaped body. Also towards the anterior extremity he noticed a longitudinal feature which gave the appearance of a buccal orifice. He also gives a good illustration of this form, and, in our minds, there is no doubt that he saw and recognised a Chilomastix.

His smaller parasite, forming variety or species B, is by consensus of opinion considered to be *Cercomonas hominis*.

We therefore have two distinct parasites called by one name, but with regard to Davaine's species A it certainly does not belong to Dujardin's genus *Cercomonas* which was founded in 1841 and was placed in his Order III, i.e., among organisms without a mouth.

Therefore *Cercomonas hominis* Davaine 1860 pro parte becomes a synonym of *Chilomastix mesnili*, but it is the genus *Cercomonas* Davaine 1860 nec Dujardin 1841 which is a synonym of the genus *Chilomastix* Alexeieff 1911.

Thirty-three years pass before we meet with another known observation upon *Chilomastix mesnili*, and this time it is described by Roos (1893) in his paper '*Ueber Infusoriendiarrhöea.*' We have been unable to consult this paper, but according to Gäbel (1914) the author states that he found in one case parasites with the following characters:—

'In der Nähe des vorderen Endes befindet sich eine Mundöffnung, die sich oft bis zur Körpermitte erstreckt. Vorn an demselben sitzen drei nicht ganz körperlange Geisseln. Sonst ist die Oberfläche glatt, und nur das hintere Ende trägt einen kurzen, unbeweglichen, stachelartigen Fortsatz.'

Gäbel gives a copy of two of Roos' drawings and they are typical of Chilomastix, and he considers that Roos was first to describe accurately a species of this genus. In another case Roos met with a typical Trichomonas with an undulating membrane. Roos, however, appears to have called both forms *Trichomonas intestinalis*; therefore *Trichomonas* Roos 1893 pro parte nec Donné 1837 becomes a synonym of *Chilomastix*, and *T. intestinalis* Roos 1893 pro parte nec Leuckart 1879 is a synonym of *C. mesnili*.

The next description is by Epstein (1893), who uses the term *Monocercomonas hominis* Grassi 1879 in his papers in the *Prager Medizinische Wochenschrift*, Nos. 38, 39, and 40, but only No. 39 contains the illustrations, of which figs. A 1, 2, and 3 were drawn from living forms by Dr. Czerny, and 4 and 5 by Professor Hatschek, after fixation by sublimate. Drawing 1 shows a pear-shaped parasite with a large deep cytostome and with two flagella; drawing 5 illustrates a similar object, and figs. A 1, 4 and 5 and fig. B 7, the illustration with the mouth and three flagella, leave the reader in no doubt that Epstein saw a Chilomastix,

as no axostyle and no undulating membrane are illustrated in these drawings, although other illustrations depict an obvious Trichomonas.

But Grassi (1888) very distinctly stated that his species *M. hominis* was a Trichomonas; therefore Epstein's *Monocercomonas* is not Grassi's, and therefore the synonym of the genus must run *Monocercomonas* Epstein 1893 nec Grassi 1879, while *M. hominis* Epstein 1898 nec Grassi 1879 becomes a synonym of *Chilomastix mesnili*.

2. *Wenyon's Discovery.* Seventeen years after the last entry in the preceding period the parasite was again observed by Wenyon (1910), who saw it in the motions of a native of the Bahamas. He remarked that in its movements there was little to distinguish it from Trichomonas except that the undulating membrane so characteristic of this genus was absent. He described the body as pear-shaped, with three long flagella directed forwards from the blunt end of the body and with a very large cytostome, the edges of which were produced into two lips, within which a flagellum or membrane could be made out displaying a constant undulatory movement. The length of the parasite varied, but the largest forms were about 15 microns long and 7 microns broad. Encysted forms, 7 by 5·5 microns, were seen, containing the characteristic nucleus, the large cytostome and some other details, but it was impossible to decide whether these cysts were reproductive or merely protective.

The flagellate was described as showing undoubted affinities with Trichomonas and Trichomastix, and was placed in the new genus *Macrostoma* Alexeieff 1909 because it agreed with *M. caulleryi* Alexeieff in all particulars, except the undulating membrane in the cytostome, which, it was just possible, had been overlooked. It was therefore called *Macrostoma mesnili* Wenyon 1910. It was further noted that the flagellate produced no ill-effects on its host, and that its presence was only discovered in the routine examination of the faeces.

It is now necessary to digress from the actual history of the parasite and to enquire what Alexeieff meant by his genus *Macrostoma*.

Alexeieff (1909) describes, but does not figure, a new genus

and a new species of flagellate which he calls *Macrostoma caulleryi*, and which he found in tadpoles and in an axolotl. The length of the parasite was 20 to 25 microns and the breadth 8 microns. It was pear-shaped, with three anterior flagella, and was characterised by possessing a very large cytostome with one border developed into a sort of lip. The nucleus was situate anteriorly.

Unfortunately the name *Macrostoma* had been used by Latreille in 1825 for a mollusc, and in subsequent years by other authors for various kinds of animals, but Latreille's genus has priority, and the other names, including Alexeieff's, lapse.

For the benefit of the Medical reader we may here point out that a generic name already in use can be discovered if it is given in Agassiz's *Nomenclatoris Zoologici* published in 1846, Scudder's *Nomenclator Zoologicus* published in 1882 or in the Zoological Society's *Index Zoologicus* No. I, 1880-1900 (its supplement by Waterhouse published in 1904) or in No. II, 1901-1910. These works are so well arranged that in a few minutes it is possible to know whether the proposed generic word is contained therein. If it is not to be found in these works then it is much more difficult to be sure that it has never been used before.

Returning to Alexeieff's *Macrostoma*, this must lapse and must become a synonym of whatever generic name is finally fixed for the parasite we are considering, and the synonym must read *Macrostoma* Alexeieff 1909 nec Latreille 1825.

In 1910, Alexeieff reported that he had obtained a flagellate from *Box salpa* which was identical with that described by Wenyon, whose parasite he had also seen in a Russian sailor suffering from cholera. At the same time he stated that he had found that the name *Macrostoma* was not available for these parasites, and suggested that they should be classified in Perty's genus *Tetramitus* which we must now, very briefly, consider.

Perty (1852) established this genus for two free living flagellates which he named *T. descissus* and *T. rostratus*, and which he illustrated as possessing *four equal anterior flagella*. Fresenius (1858) gave a brief description of *T. rostratus*, but did not advance matters, while Dallinger and Drysdale (1871) described and figured the life history of a 'Calycine Monad' which by general agreement is identified with *T. rostratus*, but they did not improve upon Perty's descriptions. Stein (1878) carefully figured the two species mentioned above, but he depicts *T. descissus* with *a long trailing flagellum*. He also described a third species which we believe to be identical with *Callodictyon triciliatum* Carter 1865.

Bütschli (1878) investigated *T. descissus*, and recorded that the flagella were of unequal length.

Klebs (1892) redescribed Perty's two original species, and added a third: *T. pyriformis*. He insisted upon the broad size of the mouth and upon the *inequality of the flagella*, of which *the fourth is always the longest*, and in two at all events of the three known species *is trailing*. Klebs' descriptions have been accepted by Blochmann, by Senn, and by Hartmann and Chagas, and are, as far as we know, the latest original writings upon species of Perty's genus *Tetramitus*. It will thus be seen that these species possess four anterior flagella, while Alexeieff's and Wenyon's parasites have only three. Again in *Tetramitus* one flagellum is trailing, while no such flagellum is described by Alexeieff or by Wenyon. Therefore we are of the opinion that it was incorrect to classify Alexeieff's and Wenyon's parasites in the genus *Tetramitus* Perty 1852. Nor had this difficulty escaped Dobell (1909), who had suggested that the name *Tetramitus* should be reserved for free living quadriflagellate organisms of the type described above, while Alexeieff (1910) proposed, that if the free living species belonging to the genus *Tetramitus* were found to be different from the parasitic, then this name should be retained for these free living forms, while that of *Chilomastix* should be given to the parasitic organisms; and, as we have shown above, there are considerable morphological differences between the parasites described by Alexeieff and by Wenyon and the generally accepted definition of a Tetramitus, and therefore the two parasites should be called *Chilomastix caulleryi* (Alexeieff 1909) and *Chilomastix mesnili* (Wenyon 1910), respectively, even though Alexeieff started the existence of the new genus without any description, and indeed without carefully differentiating it from *Tetramitus*.

Though Alexeieff did not give sufficient distinguishing features for the genus *Chilomastix* in 1910, he did so in 1911, and therefore the genus stands as *Chilomastix* Alexeieff 1911, and not 1910.

3. *Present Day*. The period which we are about to describe begins with much confusion, new genera and new species being formed for Wenyon's parasite because mistakes were made with regard to the morphology of the forms which various observers described.

Prowazek (1911) instituted the genus *Fanapepea* for a flagellate *Fanapepea intestinalis* which he discovered first in 1903 in the alimentary tract of a baboon, and later in persons suffering from Ankylostomiasis in Samoa. The organism was described and figured as a pear-shaped or rounded flagellate, with an anteriorly situate nucleus containing a small karyosome. In front of the nucleus lay two small blepharoplasts from which sprang two *anterior flagella*. Alongside the nucleus there was a large cytostome, the lips of which were strengthened by a chromatic band containing a third flagellum which arose from a third blepharoplast. This flagellum gave rise to a short cytostomic undulating membrane. No axostyle was described or depicted. In fig. 16 a twisted and grooved parasite is illustrated, which is a point of some little interest, as will be seen below. He also gives a figure of binary fission.

It seems to us that the characters of *Fanapepea intestinalis* are the same as those of *Chilomastix mesnili*, because:—

1. In fig. 8 of his paper he depicts a typical *C. mesnili* but calls it *Trichomonas nov.*, but he shows no axostyle.
2. In fig. 10 of the same paper he shows another typical *C. mesnili*, but calls it *Fanapepea intestinalis*.
3. In 1914 he, together with Werner, clearly states that *Fanapepea* is nearly identical with Wenyon's *Macrostoma*.

Therefore the name *Fanapepea intestinalis* becomes merely a synonym of *Chilomastix mesnili*.

Nattan-Larrier (1912) reported *C. mesnili*, under the term *Tetramitus mesnili*, as being present on the Ivory Coast of West Africa. He thinks that in the case which he studied the parasite may have had some pathological importance. In the discussion upon this paper Chatton said that he had seen a similar parasite in a leech *Haemopis sanguisuga* in Belfort in the year 1910.

Brumpt (1912) published an account of a case of Colitis due to *Tetramitus mesnili* (Wenyon 1910). In this paper he gave illustrations of the parasites, free and encysted. He also took exception to Alexeieff's views that the flagellate, which that observer had found in the intestines of *Box salpa*, was identical with *C. mesnili*, and called it *Tetramitus bocis*, because he said that the illustrations of the two parasites were totally different. Now Alexeieff seems to have clearly recognised the three anterior flagella and the fourth

cytostomic flagellum, and as we have been unable to see his original drawings we prefer to consider that, until further evidence is forthcoming, the parasites in man and in *Box salpa* are the same.

Alexeieff (1912) published another paper of which we have been able to obtain only some notes and some copies of his drawings of *C. caulleryi*, including those showing binary fission and cyst formation. According to these notes and drawings he considered *C. caulleryi* and *C. mesnili* as good species.

Gäbel (1914) described the same organism which he found in a German who had travelled in Tunisia. He created a new genus and species, based upon some misconceptions, using the terms *Diſämus tunensis*. He considered that the parasite was pathogenic.

Prowazek and Werner (1914) contributed some remarks upon *C. mesnili*, pointing out that it was closely related to *Fanapepea intestinalis* which Prowazek had found in Sawai, Samoa, Saipa and India. They considered that it was probable that the nuclear changes in binary fission would be found to be promitotic in character, and they gave an excellent illustration of a cyst.

Chatterjee (1915), under the name *Macrostoma*, reported the parasite's presence in the dejections of six persons suffering from intestinal troubles in Bengal.

Wenyon (1915), under the terms *Tetramitus mesnili* (*Macrostoma mesnili*), gave a further account of the parasite in question, giving the length as from 3 to 15 microns. He states that he has never seen an undoubted dividing form, but considers that the presence of specimens with two tail-like prolongations in fresh preparations may indicate simple longitudinal division. He also describes cyst formation and figures two cysts, one with a single and the other with four nuclei. When examining these drawings one is struck with the fact that the nuclei do not appear to be typical of *Chilomastix*, and, as we shall see later, Wenyon finally decided that they were not *C. mesnili* cysts, but those of an amoeba.

Mackinnon (1915), in a paper mainly dealing with the genus *Embadomonas* (which appears to us to be the same as Wenyon and O'Connor's new genus *Waskia*), drew attention to its close affinity to *Chilomastix* Alexeieff 1911.

Da Fonseca (1915 and 1916) reported that *C. mesnili* existed in Brazil, and also added to the genus several new species derived from mammals.

Fantham and Porter (1915) reported its presence in the motions of soldiers from the Mediterranean War Zone.

Archibald, Hadfield, Logan and Campbell (1916) found it to be present four times in the large number of motions of soldiers which they examined during the Gallipoli campaign.

Chatterjee (1917) stated that he had found it eighteen times in stools of chronic dysentery in Bengal.

Wenyon (1917) found it in films of faeces from the Philippine Islands and from Panama; and Wenyon and F. W. O'Connor (1917), in their excellent paper upon '*Problems affecting Intestinal Protozoal Infections*,' noted that the parasite is often flattened and leaf-like and that its long-drawn-out flattened posterior extremity may be twisted and folded in a variety of ways, thus producing grooves and spiral turns. This is an important observation to which we shall return later. They note that binary fission probably takes place but has not yet been observed. They lay great stress on the cysts, which they state can be found in the formed stools, and they point out that the four-nucleated cyst illustrated in Wenyon's (1915) paper does not belong to *C. mesnili* but to *Entamoeba nana*, a mistake which everyone who has worked at this small organism knows is very liable to occur even when one is well acquainted with some of the stages of the cyst—and it must be remembered that *C. mesnili* is generally accompanied by a number of other parasites (*vide* the list given in the Introductory Section of this paper). They also state that *intermission* of the presence of the parasite in the stools is not so marked in the case of *C. mesnili* as it is with other intestinal parasites.

From this history it will be evident that a considerable number of observations have been made with regard to the parasite in question, but unfortunately a great deal of confusion has arisen owing to mistakes as to the number of anterior flagella, the presence or absence of the cytostomic flagellum and its action, while much more confusion has arisen as to the cystic form of the parasite and its pathogenicity. Moreover, the names used for the same parasite are numerous and confusing, and yet it is essentially *a common human intestinal flagellate*, and therefore we consider that any contribution, however small, which attempts to clear up some of these points may be of use to workers in the Tropics.

III. GEOGRAPHICAL DISTRIBUTION

Chilomastix mesnili appears to be widely distributed all over the world.

So far as we know, it has been recorded in man from the following places:—

Europe:—France, Germany, Russia, Salonika War Area and Turkey.

Asia:—India and the Philippine Islands.

Oceania:—Samoa, Sawai and Saipa.

America:—Brazil, Panama and the West Indies.

Africa:—The Ivory Coast, Tunisia, Egypt and the Anglo-Egyptian Sudan.

IV. ZOOLOGICAL DISTRIBUTION

So far its principal host is man, in whom it would appear that all races will be found to be infected, or susceptible, as it is recorded in Europeans, Natives of India, Natives of Samoa, Negroes and Arabs.

It has also been found in a baboon, and in a fish *Box salpa*, but, with regard to this last host, Brumpt has raised the doubt as to whether it is not a separate species, for which he suggests the name *Chilomastix bocis*.

V. THE LIVING PARASITE

Chilomastix mesnili in the fresh condition in the faeces is usually pear-shaped (figs. 1-4, 6-7 and 9-11), but, at times, it may assume other forms, being fusiform (fig. 5) or roundish (fig. 14). It measures on an average, and excluding the very small developmental forms, some 11 to 18 microns in length, if the caudal appendix is included, and some 1·6 to 4·0 microns less if this is excluded. In breadth it attains some 6 to 8 microns in greatest measurement.

With regard to its orientation, the anterior end is the broad portion of the pear-shaped animal, and is characterised by the presence of the nucleus and by the origin of the three flagella. The ventral surface is that on which the cytostome opens.

When fresh faecal matter is examined the parasite may be

readily seen dashing quickly about among the débris, but it is not until this rapid motion has abated that it is possible to see that it moves with its broad extremity placed anteriorly.

When moving still more slowly it is possible to see that this anterior end is provided with three free equal anterior flagella. These flagella as a rule are not longer than the body, and work by lashing from side to side.

Rotatory movements can also be observed, as can a peculiar motion of the anterior portion of the body, which appears to consist of a dorsal twisting of the antero-ventral part, while the posterior part of the body is not affected. It is a quick movement, and gives one the impression that the antero-ventral portion is quickly pulled dorsally for a short distance and then quickly returned to its original position. If the parasite is fixed while performing this dorsal twist it appears as represented in fig. 15.

This twist naturally gives rise to the appearance of lines and grooves running more or less diagonally backwards across the body.

There is a very evident and large cytostome (figs. 3 and 4) situate anteriorly, and extending posteriorly a considerable distance into the body of the flagellate. This cytostome possesses very distinct lips, which open (figs. 4 and 5) and close (figs. 8 and 13). There is also a movement of the nuclear or inner lip which resembles that of a short undulating membrane. It would appear to be due to the action of a fourth or posterior flagellum (figs. 6 and 11) lying in the inner lip and pulling outwards the inner wall of the cytostome into the form of a bow-shaped membrane (figs. 7, 9, 10 and 11), which returns to its original position when the flagellum has ceased to contract. There can be little doubt that the action of this membrane helps the flagellate to convey motionless food particles to the cytostome. It seems to us that these thickened mobile margins (figs. 4 and 5) of the lips of the cytostome are of great service in enabling the flagellate to secure a firm hold on its food. To exemplify this we may quote an incident which we have noticed. We have seen a parasite swim up to a chain of bacilli evidently much too long to be completely engulfed by the cytostome. This chain was promptly seized by the lips of that opening and then the parasite set to work to try to swallow its prey, but while so engaged a passing flagellate, doubtless attracted by the commotion, stopped

and seized hold of the other end of the chain with its cytostome, whereupon a struggle ensued which demonstrated in an admirable manner the movements of the flagella, the lips of the cytostome and the caudal appendix, as it lasted for some ten minutes. The second comer appeared never to get a firm hold of the chain, which was being jerked about at the time that it arrived, because it was repeatedly thrown off, but the first one had a firm grip, and it appeared to us, watching the struggle, that the thickened lips helped that hold considerably, as they appeared to be very strong; but, be that as it may, the second flagellate gave up the game and went away, leaving the first claimant alone with its gargantuan feast.

The food of the parasite appears to be the micro-organisms to be found in the intestinal contents. We have never observed red blood corpuscles in its interior.

It seems to be readily killed in the adult condition by exposure to cold, dryness and light.

We have been unable to cultivate the flagellate on ordinary laboratory media aërobically or anaërobically.

In sand we have merely obtained some organism not unlike that described by Ross and Thompson (1916), by the Thomsons (1916), and by Chatterjee (1914).

We have met with an allied species in white rats, originally brought from England, but bred for several years in the Sudan, and also in the Sudan Gerbil (*Gerbillus pygargus*).

VI. MORPHOLOGY

We have studied the structure of the parasite entirely by films made from faecal matter, as we have been unable to obtain a post-mortem in a case known to harbour the organism. The flagellates were killed by osmic acid, fixed in Schaudinn's fluid and stained by iron haematoxylin.

In specimens so prepared the most striking object (fig. 1) is the large roundish, oval, or slightly irregular nucleus, which measures some 2·8 microns in its greatest diameter and lies in close proximity to the anterior extremity.

As a rule the nucleus stains homogeneously, but in well decolourised specimens (fig. 1 *a*) the rim is more darkly tinted and

there is a lighter centre. There are often two or three darker and thicker spots of variable size and appearance situate on this rim, but these may be absent and there may be one large, more or less rounded, central karyosome, or two medium-sized karyosomes lying in the central portion and not attached to the rim. In other instances three small karyosomes may be seen.

Situate anteriorly or antero-laterally to the nucleus two chromatic particles can usually be readily seen (fig. 5), but, when careful decolourisation has been conducted, it will be observed that there are really three (fig. 2) chromatic particles or blepharoplasts. One of these is situate anteriorly, and gives rise to *two of the anterior flagella*; the second lies externally and is the origin of the *third anterior flagellum* and of the chromatic outer rim of the cytostome which, as we shall see later, is probably homologous to a '*Parabasal.*' The third and small blepharoplast lies posterior to the first and internal to the second, and gives rise to a fourth or *cytostomic flagellum* (fig. 2).

We have failed to observe any trace of a rhizoplast.

Situate externally to the nucleus, and extending considerably posterior thereto, lies the large cytostome (figs. 3 to 13). It measures some 2·8 microns in length by some 1·2 microns in breadth when widely open, but, of course, its dimensions vary considerably, and its general characters can be best judged by an examination of the figures just quoted.

If figures 3, 4, 5 and 13 are examined it will be seen that the margins of the mouth show well defined siderophilous thickenings, which in fig. 5 appear to run continuously from one blepharoplast, all around the cytostomic opening to the other blepharoplast. This condition has been noted by many observers, and has produced much misconception as to the nature of these lips.

In this quiescent condition it will be noted that the outer lip is, as a rule, distinctly wavy, while the inner or nuclear lip is straight (figs. 3, 4, and 5).

When more carefully studied, even in this quiescent condition, it can be seen that the thicker and more important siderophilous rim runs down the whole length of the outer lip, and curving around the posterior end of the cytostome ascends the inner lip for some distance, as is shown in figs. 3 and 6. It now stops, but its outer

aspect is overlapped by the nuclear siderophilous rim, as can be easily seen when the two are separated as in figs. 3 and 6, but cannot be observed in figs. 5 and 13, in which it is impossible to say where one structure begins and the other ends.

The inner or nuclear siderophilous rim appears to us to take the black stain less deeply (fig. 6) than the outer rim. It starts from the third blepharoplast (fig. 2), or from a fusion of the first and third when this latter is absent, and runs posteriorly down the inner or nuclear lip of the cytostome (fig. 3), being placed externally to the anteriorly running prolongation of the external rim (fig. 6). It is capable of contraction and of being displaced outwards (figs. 6, 9, 10 and 11), and when this takes place it raises a bow-shaped membranous expansion (fig. 7) from the inner aspect of the cytostome. This movement may be carried across the mouth so that the membrane touches, or almost touches, the outer lip and so temporarily divides the mouth into an anterior and a posterior opening (fig. 8). When this takes place it is seen (figs. 6 and 11) that not merely is this rim attached to a blepharoplast, but it is also attached near to the postero-internal angle of the cytostomic opening.

When its contraction is over it returns to its original position in the inner rim of the mouth, and by this alternate movement produces a flickering and undulating movement in the cytostome very reminiscent of the undulating membrane of a Trichomonas.

If the specimen is subject to somewhat rough treatment this internal siderophilous rim can be seen hanging in the cytostome (fig. 12) and then produces the appearance of a free cytostomic flagellum, but, should the treatment be even rougher, it may be torn from the cytostome and appear as a short free fourth anterior flagellum as figured by Gäbel.

These artefacts are extremely interesting, showing that this internal rim is really a fourth posteriorly directed flagellum which penetrates into the cytoplasm, and is capable, on contraction, of raising a membrane, homologous to an undulating membrane in such an organism as a typical Trichomonas, such as is depicted in fig. 16.

If this is admitted we have still to explain the outer siderophilous rim, and this appears to us to be homologous to the '*Parabasal*' or chromatic thickening at the base of the undulating membrane of the *Trichomonas*, as can be seen by an examination of fig. 16. It will, however, be observed that there are two great differences between the

undulating membrane or fourth flagellum of the *Trichomonas* and its parabasal and the siderophilous rims of the cytostome of the *Chilomastix*, and these are:—

1. Both organellae are dorsal in *Trichomonas* and ventral in *Chilomastix* (assuming that the cytostome marks the ventral surface in both flagellates).
2. In *Trichomonas* the flagellum is situate external to the parabasal, while in *Chilomastix* this position is reversed.

These two differences appear to us to be co-related. We believe that in development and evolution the posterior flagellum and the parabasal growing backwards from the blepharoplasts of the *Trichomonas* for some unknown reason took the dorsal side of the nucleus, and meeting with no obstruction were able to grow backwards through the whole length of the flagellate (fig. 16). In *Chilomastix* for some unknown reason they grew down the ventral side, and in so doing their relationship to one another would be exactly the reverse to that found in *Trichomonas*, viz., the parabasal would be external and the flagellum internal. Moreover, they would meet with the cytostome round which the parabasal curves, thus checking the growth of the flagellum. The curving of the parabasal round the cytostome reminds us of the way in which the same organella winds round the axostyle in *Devescovina*. We therefore see a homology between the flagellum in the inner lip of the cytostome of *Chilomastix* and that in the undulating membrane of a *Trichomonas*, and between the siderophilous thickening in the outer lip of the cytostome of *Chilomastix* and the parabasal of a *Trichomonas*.

We now turn to ask ourselves whether this parabasal has any other function besides that of being a thickening to the outer lip of the cytostome. We have already referred to the curious twisting to and fro movement observed in the anterior portion of the body of the parasite at times, and we believe that it is this movement which produces the twisted appearance depicted in fig. 15.

We suspect that this movement is brought about by the action of the parabasal, and our reasons for this suggestion, for it is nothing more than a suggestion, are:—

1. Because we can find no other structure which can produce this movement.
2. Because the parabasal is often found to be wavy (figs. 3, 4, 5 and 6), as though caught in the act of movement.

3. Because of its persistence in cysts indicating some important function.

The twisted condition of the parasite when caught performing this movement gives rise to the appearance of a groove sloping diagonally and posteriorly along or across the back portion of the body (fig. 15).

The cytoplasm of the flagellate is colourless, finely granular and contains, as a rule, a number of food vacuoles (fig. 1). No contractile vesicle can be seen.

The periplast is thin, apparently elastic, and without any marked thickenings. It is prolonged posteriorly into the so-called 'caudal appendix or tail,' which may (fig. 1) or may not (fig. 6) contain a slight amount of cytoplasm at its base. It is this appendix which gives the pear-shape to the flagellate, but it is not always present, and then the shape of the parasite is rounded (fig. 14). It may possibly function as an organ of temporary attachment of the flagellate to some neighbouring structure.

VII. DEVELOPMENT

We have met with two forms of development, viz., *binary fission* and *cyst formation*. We have never seen any sign of multiple fission, but we have come across a number of small forms.

Binary Fission. Judging by the rarity of these forms in the faeces, as experienced by ourselves and other observers, we are inclined to the view that this form of reproduction is intended for the propagation of the parasite in its host, and we think that until the bowel in a case of infection can be studied it will be unlikely that any complete series of the changes undergone by the parasite will be obtainable. At all events, we have been unable to find any such series, and the few dividing forms which we have met with have produced exceedingly poor photographs. We therefore wish such views as are set forth here to be considered as merely tentative.

Starting with the non-dividing nucleus, we have the condition of affairs represented in fig. 2, viz., the nucleus with its chromatic apparatus and three blepharoplasts with their attached three anterior flagella, one parabasal, and one posterior flagellum. The earliest stage of mitosis which we have observed is that set forth in fig. 17,

in which the nucleus seems to be gathering its chromatin into two masses, and there are only two distinct blepharoplasts with four flagella. The mouth is exceedingly indistinct, although the parabasal is just visible.

In the next stage (metaphase) which we have met with, the chromatin particles are arranged in an equatorial plate consisting of four chromosomes. Starting from the left there is a blepharoplast with two flagella attached; then comes a second blepharoplast with one flagellum and also with a chromatic line running over the length of the nucleus and passing on a level between the anterior and the second chromosome to reach the right end of the nucleus, where it joins with another blepharoplast also carrying a flagellum. This chromatic line appears to indicate a *paradesmose*. Just posterior to this right-handed blepharoplast there lies a fourth blepharoplast giving rise to two flagella, while posterior again some remains of the mouth margins can be distinguished.

A further stage (anaphase) seemed to be indicated by a form which showed two chromatin particles (chromosomes) at each end of the nucleus. Just outside each pole (right and left) of the nucleus there lay a chromatic particle connected by fine lines to the intranuclear chromosomes, while these in their turn were also connected by fine lines, thus making a spindle.

The difficulty which we have with this stage is that whereas the equatorial plate had four chromosomes, each end of this nucleus had two (and no more) chromosomes, as the specimen was well decolourised and the four chromosomes were seen very clearly. The blepharoplasts were now situate some little distance from the nucleus and showed the same general arrangement as in the preceding specimen, except that there was no paradesmose, and that the first blepharoplast gave off only one, and not two flagella as mentioned above. In the penultimate stage (late anaphase) which we have seen, the chromatin particles were gathered into two fair-sized lumps more or less fused together at either end of the nucleus, which was considerably elongated, while the blepharoplasts and flagella were a replica of the second division stage described above.

The last stage (telophase) which we have observed is that depicted in figs. 18 and 19, in which it will be observed that the parasite is much elongated and that the two nuclei lie at opposite

poles, and that two or three blepharoplasts give rise to three flagella. A mouth is visible near each nucleus in these specimens, but is only partially in focus in the illustrations.

These are the only stages which we have observed. They are obviously incomplete, and they are peculiar: for example, we can offer no explanation of the apparent reduction in the number of the chromosomes, and we must leave the clearing up of this and many other points in this mitosis to future investigations.

Cyst formation. We have seldom found the cysts in the faeces, and then only in cases of fairly severe diarrhoea. The typical cyst (figs. 22 and 23) usually measures some 7 by 5·6 microns, and is now extremely easy to identify, being somewhat egg-shaped (figs. 22 and 23), that is to say, having its anterior end narrower than its posterior. The cyst wall (figs. 21, 22 and 24) is separated from the parasite by a clear space. In its youngest stages (figs. 20 and 21) it is possible to discern the nucleus, and a blepharoplastic mass (situate anteriorly) from which run two chromatic lines which appear to be the mouth margins, viz., the parabasal and the posterior flagellum, and sometimes other rather vague siderophilous lines running from front to back which represent remains of the anterior flagella. Sometimes the nucleus looks as though it was composed of four portions closely joined together.

The only development which we have seen to take place in the cyst has been the disappearance of all the siderophilous lines, leaving the cyst with a very distinct nucleus containing a single large karyosome (fig. 24; it is also well shown in fig. 21 which represents an earlier stage), and surrounded by a clear space but without any evident membrane. Such a nucleus appears to us to be of the nature of a '*Protokaryon.*'

Just outside the nucleus, and often lying in close contact with it, is a chromatic mass (figs. 22 and 24), and, as a rule, one or two other small siderophilous particles can be seen lying in the cytoplasm.

We have seen signs of division twice; first it was merely in the nucleus (fig. 25), which consisted of two chromatic poles connected by means of a thick chromatic rod. This appeared to us to be an early stage of a promitotic division. Outside this dividing nucleus lay the usual chromatic or siderophilous mass, which was elongated in the same direction as the dividing nucleus.

The second showed two nuclei embedded in granular protoplasm (fig. 26).

These cysts are extremely difficult to photograph, but we have preferred to give untouched photographs, however imperfect, rather than drawings. We look upon the cysts as the means by which the parasite changes its host.

Young forms. We have met with numerous small forms, of which the smallest we have seen (fig. 27) measured some 4·2 by 2·2 microns. It possessed a well marked nucleus and three flagella, but we were unable to make out any mouth, and it contained no micro-organisms. In the photograph some organisms are lying over but not inside the parasite.

The next larger type of flagellate is depicted in fig. 28, in which a well marked mouth can be seen, and the development can proceed through such a type as that depicted in fig. 29 till by growth it reaches the adult form.

We have no evidence as to where these young forms come from, though judging by the size and the absence of the mouth it is possible that they come from the cysts.

Such are the few, fragmentary and unsatisfactory details which we are able to give of the development of *Chilomastix mesnili*, and therefore we will now pass on to consider its classification.

VIII. CLASSIFICATION

As the protozoal organism which we have described above possesses permanent flagella which serve as organs of locomotion and also assist in the capture of food, it belongs to the Class *Mastigophora* Diesing 1866, which is divided into sub-classes as follows:—

DIAGNOSTIC TABLE OF THE *Mastigophora*

A. *Body inflated with gelatinous substance*	Sub-class 1 *Cystoflagellata* Haeckel 1873
B. *Body not so inflated*:—	
I. Periplast markedly thickened with two flagella arising in the middle of the body; one trailing and one lying in the transverse groove	Sub-class 2 *Dinoflagellata* Bütschli 1885

II. Periplast thin with a variable number and arrangement of flagella...	Sub-class 3 *Euflagellata* Cohn 1887

The parasite in question obviously belongs to Cohn's *Euflagellata*, which may be classified into the following orders:—

DIAGNOSTIC TABLE OF THE *Euflagellata*

A. *Chromatophores often present:*—	Order 1
I. With cellulose envelope	*Phytomonadina* Blochmann 1895
II. Without cellulose envelope:—	Order 2
a. Small forms without oesophagus or vacuole system	*Chromomonadina* Klebs 1892
b. Large forms with oesophagus and vacuole system	Order 3 *Euglenoidina* Bütschli 1884
B. *Chromatophores absent:*—	Order 4
I. Amoeboid forms in which the food is captured by pseudopodia	*Rhizomastigina* Bütschli 1884
II. Non-amoeboid forms in which the food is usually captured by flagella	Order 5 *Protomonadina* Blochmann 1895

Our parasite certainly belongs to Blochmann's *Protomonadina*, which, with the above definition, includes the orders Binucleata and Polymastigina, and can be sub-divided as follows:—

DIAGNOSTIC TABLE OF THE *Protomonadina*

A. *No tendency to bilateral symmetry in undividing forms.* Anterior flagella vary from one to many, in addition to which a trailing flagellum or undulating membrane may be present ...	Sub-order 1 *Monozoa* Hartmann and Chagas 1911
B. *More or less tendency to bilateral symmetry in undividing forms* as shown by the arrangement of the flagella, the duplication of the axostyles, sometimes of the nucleus, and more rarely of the cytostome. Undulating membranes absent	Sub-order 2 *Diplozoa* Hartmann and Chagas 1911

As the parasite in question does not show any signs of bilateral symmetry it belongs to the Sub-order 1 *Monozoa*, which is classifiable into the following families:—

DIAGNOSTIC TABLE OF THE *Monozoa*

A. *One flagellum present* :—	
I. With a collar	Family 1 *Craspedomonadidae* Stein 1878
II. Without a collar :—	Family 2
a. Blepharoplast not separate from the nucleus	*Oicomonadidae* Senn 1900
b. Blepharoplast separate from the nucleus	Family 3 *Trypanosomidae* Doflein 1901
B. *Two flagella present* :—	
I. Both anterior :—	
a. Unequal	Family 4 *Monadidae* Stein 1878 emendavit Senn 1900
b. Equal	Family 5 *Amphimonadidae* Kent 1880 emendavit Bütschli 1884
II. One anterior and one trailing flagellum :—	
a. Trailing flagellum free :—	Family 6
1. In horny sheath and with lip or proboscis-like process	*Bikoecidae* Stein 1878
2. Without sheath or process ...	Family 7 *Bodonidae* Bütschli 1884
b. Trailing flagellum in part attached to the body (except *Tricercomonas* with three anterior flagella)	Family 8 *Cercomonadidae* Kent 1880 emendavit Bütschli 1884
C. *Three to six anterior flagella* with or without one trailing flagellum (except *Embadomonas* with one anterior and one cytostomic flagellum)	Family 9 *Tetramitidae* Kent 1880
D. *Numerous anterior flagella*	Family 10 *Callimastigidae* Da Fonseca 1915

IX. TETRAMITIDAE

It is sufficiently evident that the organism we are considering comes under the heading Tetramitidae, which may be defined as follows :—

'*Protomonadina* with three to six anterior flagella (with the exception of *Embadomonas* which has only two flagella, one

anterior and one lying in a large cytostome with siderophilous lips), with or without a rhizoplast, with or without one trailing flagellum which may or may not form an undulating membrane, and with or without an axostyle and a cytostome.

Habitat: Free living and parasitic.

Type genus: Tetramitus Perty 1852.'

It is not possible in Khartoum to obtain a full literature, and the following list of genera which come under this family merely comprise *those with which we are acquainted*, and therefore does not pretend to be a complete list.

With this proviso and because so many human parasites are included in this family which, with the above definition, represents not merely Saville Kent's *Tetramitidae* but also his *Trimastigidae*, we consider that it is necessary briefly to consider every genus which at any time has been classified in these families.

Our reason for this is because we wish clearly to define each genus which is properly classifiable in the Tetramitidae, in order to do away with the existing confusion and in order to prepare a diagnostic table with a view to defining clearly the position of the genus *Chilomastix*.

We shall not consider the new genus *Tricercomonas* Wenyon and O'Connor 1917 as it appears to have very definite affinities with the genus *Cercomonas*, notwithstanding its three anterior flagella, and therefore has no place in the Tetramitidae; but with regard to some thirty-four genera which from time to time have been placed in one or other of Saville Kent's families we have the following remarks to make:—

GENUS 1. *Chloraster* Ehrenberg 1836:—Ehrenberg (1836) instituted this genus for *C. gyrans*, which having its cytoplasm coloured green was removed from the *Tetramitidae* by Bütschli and was subsequently classified by Blochmann in the family *Polyblepharididae* Dangeard 1888 which belongs to his order *Phytomonadina* and therefore does not further concern us.

GENUS 2. *Trichomonas* Donné 1837. In his work '*Recherches microscopiques sur la nature du mucus*,' Donné (1837) described and figured an organism which he found in innumerable quantities in vaginal mucus.

He considered that it possessed a single flagellum which at times was bifurcated distally, a series of three to five cilia with very rapid

rotatory movement, and that at times it was elongated posteriorly into a tail. The name was first spelt Tricomonas, but afterwards altered to Trichomonas.

Dujardin (1841) described *T. limacis* from *Limax agrestis* in much the same terms, and so did Perty (1852) with regard to *T. batrachorum*, though he depicted the axostyle, but Stein's figures of Perty's organism show clearly the three anterior flagella, the undulating membrane, the posterior free flagellum, the axostyle, the nucleus and the cytostome, and in this way was laid the foundations upon which the main features of the genus were placed.

Returning now to the type *T. vaginalis*, this was re-studied in 1884 by Blochmann, who illustrated the three anterior flagella, the undulating membrane, the axostyle and the nucleus, but in the same year Künstler produced a much better illustration showing the three anterior flagella taking their origin from a blepharoplast from which the undulating membrane also arose, while this shows a trace of a parabasal. The nucleus is also represented, while the axostyle shows exceedingly clearly. He also saw the cytostome. Bensen (1910) figured two blepharoplasts, one of which is connected with the nucleus by means of a rhizoplast, and he also gave an illustration of a cyst. Thus the type species *T. vaginalis* was brought into line with the results of researches upon the species found in animals, of which a number have been carefully described and drawn by Dobell, Alexeieff, Martin and Robertson, Kuczynski, and by Kofoid and Swezy.*

With regard to the Sudan, there have been strikingly few observations upon species of this genus. Stevenson (1911) figured *T. batrachorum* from *Bufo regularis*. Since then we have observed several species in animals which we hope to describe in a future paper.

We have only very rarely seen *T. intestinalis* (Leuckart 1879) in man, and this is very curious, considering the very large number of

* Since writing the above, we have had the good fortune to procure a copy of Künstler's paper on *Trichomonas vaginalis* in which he states: 'Le *Trichomonas vaginalis* présente quatre flagellums antérieurs.' In this country it is almost impossible to obtain specimens of this organism from the vagina, but by good fortune we obtained a few specimens from the urine of one person, and certain of these had undoubtedly four flagella; which facts point to the type of the genus *Trichomonas* as being in reality a *Tetratrichomonas*, unless there are two different parasites in the vagina, viz., a *Trichomonas* and a *Tetratrichomonas*.

examinations which we have made during the last four years. We have so far only once met with *T. vaginalis* in the Sudan.

GENUS 3. *Pyramimonas* Schmarda 1849:—This genus was founded by Schmarda for *P. tetrarhynchus* of which drawings are given by Stein who called it *Pyramidomonas*.

It was placed in an appendix to the Chlamydomonadidae by Bütschli, while Doflein classifies it in Dangeard's Polyblepharididae, which belongs to Blochmann's order *Phytomonadina*. It can therefore be excluded from the *Tetramitidae*.

GENUS 4. *Tetramitus* Perty 1852. This genus is of importance because it is the type of the family which we are studying, and because *considerable confusion* has arisen not merely in medical but also in zoological literature as to its exact definition.

The history of the genus has, however, already been described in the section dealing with the history of *Chilomastix mesnili*, while its definition will be given later on in this paper, and therefore we merely draw attention to what is known with regard to the life history.

Perty described binary fission, and Dallinger and Drysdale confirmed the binary division mentioned by Perty, and also reported sexual conjugation followed by encystment and minute spore formation from which the young animalcules arose.

GENUS 5. *Callodictyon* Carter 1865. There is a great deal of confusion about this genus. In the first place, German authors spell it Collodictyon, which appears to be wrong. In the second place, it was established for *C. triciliatum*, which according to Bütschli is the same organism as that described by Stein as *Tetramitus sulcatus*. Klebs disagrees with this, but only on the ground that *T. sulcatus* has four and not three flagella, while Carter might easily have missed one flagellum.

Blochmann considers that they are one and the same species, and it must be admitted that the representations of the two species admit of this explanation.

Blochmann defines the genus as follows:—

> 'Colourless, anteriorly broad, posteriorly broad or drawn out finely, with a deep ventral longitudinal furrow, and four anteriorly springing flagella.'

To which may be added that two of the flagella are longer than the other two.

It obviously belongs to the Tetramitidae.

GENUS 6. *Tetraselmis* Stein 1878:—The only species belonging to this genus was described by Carter in 1858 as *Cryptoglena cordiformis*, but Stein considered that it should form a separate genus, hence the origin of *Tetraselmis*; but Diesing had already created the genus *Carteria* in 1865 for this species; therefore Tetraselmis becomes merely a synonym of *Carteria*. The animalcule possesses green endoplasm and is classified by Blochmann in the family *Chlamydomonadina*, which belongs to his Order *Phytomonadina*, and is therefore excluded from the Tetramitidae.

GENUS 7. *Monocercomonas* Grassi 1879. This genus was subdivided by its author into four sub-genera, which, thanks to Stiles' valuable researches, are easy to understand. They are:—

(*a*) *Monocercomonas*. Stiles has shown that the type of this sub-genus must be *M. coronellae*, found parasitic in *Coronella austriaca*, and it is the only species left, as Grassi himself eliminated five out of his seven species, leaving only *M. hominis*, which is recognised to-day as a Trichomonas, and *M. coronellae*. Doflein, however, considers that this latter species is the same as Dobell's *Trichomastix serpentis*.

(*b*) *Trichomonas*. We have already considered this genus.

(*c*) *Retortamonas*. According to Stiles, this is the same as Grassi's genus *Plagiomonas*. The type and only species is *R. gryllotalpae* Grassi 1879. Doflein considers this sub-genus to be a *Bodo*, and certainly, judging by Grassi's figures, it does not appear to belong to the Tetramitidae.

(*d*) *Schedocercomonas*. This sub-genus originally included four species, of which *S. gryllotalpae* and *S. melolonthae* were transferred by their author in 1881 to *Monocercomonas* under the single name *M. insectorum* Grassi 1881. The third species *S. caviae*, found in the guinea-pig, is unrecognisable, as its diagnosis rests upon the words 'quasi senza corda'; moreover Stiles was unable to trace a reference to it later than its origin in 1879, and therefore it may be eliminated. The fourth species is the well-known *Herpetomonas muscaedomesticae*.

Thus all the species of Grassi's sub-genera disappear, and the genus *Monocercomonas* Grassi 1879 is left with only two species, viz., *M. gryllotalpae* and *M. melolonthae*, which are considered by their author to be identical, and, of the two, priority is given to the former, which is believed to be the same organism as *M. cetoniae* Jollos 1911 but different from *M. bufonis* Dobell 1909. It has been carefully studied by Mackinnon, and will be defined later.

A flagellate has been found by Chatterjee in man in choleraic and diarrhoeal motions which he refers to this genus, but there is no axostyle depicted in his drawings, and it is uncertain to what genus it belongs.

GENUS 8. *Trimastix* Saville Kent 1880. This genus was created for *T. marina* Saville Kent 1880 found in salt water, which is the only and hence the type species.

It possesses three flagella, one of which is directed anteriorly and two posteriorly, of which one is free while the other is retained in the angle or depression formed between the body and a membranous non-vibratile expansion of the right lateral border.

No cytostome was observed, but the angle or depression mentioned above is in all probability this organella, though this cannot be stated with any certainty as the organism has never been investigated by modern methods.

It is peculiarly interesting from our present point of view, as it is a free living organism with a flagellum retained in part of its course in what is apparently a cytostomic depression.

GENUS 9. *Dallengeria* Saville Kent 1880. It was created by Saville Kent in 1880 for the free living species *D. drysdali* Saville Kent 1880, and is so remarkably like genus (13) *Elvirea* created by Parona in 1887 for a parasitic species found in the intestine of *Ciona intestinalis* Linnaeus, that it seems to us unnecessary to differentiate these genera, although the species may be different, until they are examined by modern methods, especially as the former was found in animal macerations.

GENUS 10. *Cimaenomonas* Grassi 1881 was instituted mainly for *C. batrachorum* (Perty 1852) which was considered by Perty and others to be a Trichomonas, to which genus Grassi's drawings, according to Stiles, clearly show that it belongs. *Cimaenomonas* therefore becomes a synonym of Donné's *Trichomonas*.

GENUS 11. *Polymastix* Bütschli 1884. This genus was created by Bütschli for the organism named *Trichomonas melolonthae* by Grassi in 1882. It has been carefully re-examined by Hamburger (1911) and by Mackinnon (1913), and is now known to possess four flagella arranged in two groups, each belonging to a separate blepharoplast between which lies a cytostome. A vesicular nucleus lies behind the blepharoplast, from which an axostyle arises. The

characteristic feature of the genus is the peculiar condition of the periplast, which is raised into numerous longitudinal ribs or folds.

Unfortunately Künstler stated that *P. melolonthae* possessed six flagella. This naturally led to much confusion until Alexeieff formed the genus *Hexamastix* as will be mentioned below.

GENUS 12. *Trichomastix* Blochmann 1884. This genus was created by Blochmann for the parasite known as *Trichomonas lacertae* Bütschli, because its fourth flagellum, instead of forming an undulating membrane as in the case of a typical Trichomonas, remains free as a trailing flagellum.

Unfortunately it is necessary to alter this generic name, because it was originally introduced by Svellen van Vollenhoven in 1878 for a genus of the Hymenoptera with a type species *Trichomastix polita* v. Vollenhoven 1878, and as such was published on page 160 of Volume XXI of the *Tijdschrift voor Entomologie*, together with an illustration of the insect on Plate 9, fig. 4.

Raillet (1893) spelt the name Trichomast*y*x, but this slight change in spelling is not sufficient alteration to hold good.

We had written in this manuscript what we considered to be a suitable name when we received Kofoid and Swezy's paper, in which they suggest the name *Eutrichomastix*. This is a sufficient alteration and must stand, though we would have preferred some such term as *Axomastix*, thus making a greater difference in the nomenclature between the Hymenopteron and the Protozoon.

Besides the type, quite a number of species are known to belong to this genus, thanks to the labours of Dobell, Mackinnon, Martin and Robertson, Alexeieff and Parisi. We have met with several species, some probably new, in various animals in the Anglo-Egyptian Sudan.

GENUS 13. *Elvirea* Parona 1887:—As already stated above we believe that this genus should be absorbed into genus (9) *Dallengeria*.

GENUS 14. *Costia* Leclerq 1890:—Leclerq formed this genus for the organism known as *Bodo necator* Henneguy 1884. Leclerq's name cannot stand, as the same word was used by Kirchner (1867) for a Hymenopteron. In this instance a change of name is most useful, because Senn (1900) instituted the genus *Costiopsis* for the parasite called by Weltner (1894) *Tetramitus nitschei*, which Moroff's (1903) researches proved to be the same as *Costia necatrix* synonym *Bodo necator*. Therefore the name *Costia* is excluded.

GENUS 15. *Costiopsis* Senn 1900. As just explained above, this name takes the place of *Costia* Leclerq. Unfortunately, situate in Khartoum in time of war, we have been unable to refer to the original papers of Henneguy, Leclerq, Nitsche and Weltner and of Moroff, and have obtained our knowledge of these organisms from Neresheimer's work and the writings of Senn.

The principal characters of the genus are that the body is dorso-ventrally flattened and asymmetrical, and that the true morphological anterior end is about the middle of the left lateral border. On the ventral surface there is a depression which serves as a suctorial disc which becomes funnel-shaped in front, and in this the cytostome lies, near the morphological anterior end, where also arise the four flagella, of which two are thick, long and trailing, while the other two are fine and short and remain in the mouth cavity. In the middle of the body there is a vesicular nucleus with a contractile vacuole on either side. There is no undulating membrane and an axostyle is not mentioned, and is therefore assumed to be absent.

The organisms can swim about in water, but are essentially ecto-parasites of the skin of young gold fish, carp, &c.

GENUS 16. *Devescovina* Foà 1905. The Monozoa by means of Da Fonseca's family *Callimastigidae* appear to be very closely related to Leidy's family *Trichonymphidae*, but, be this as it may, we quite agree with Kofoid and Swezy that the genus *Devescovina* should be removed from this family and should be included within the *Tetramitidae* near *Eutrichomastix*, and we note that Janicki (1915) appears to have done this in a paper which we have been unable to see.

The genus *Devescovina* was created by Foà (1905) for a flagellate organism which she obtained from the Neuropteron *Calotermes castaneus* Burmeister found in Honolulu.

The parasite, which is more or less oval in form, possesses three equal anterior flagella which spring from an anteriorly situate blepharoplast. There is also a long trailing flagellum, an anteriorly situate nucleus, and a well marked axostyle, around the anterior portion of which the thick parabasal is wound.

It would appear from Kofoid and Swezy's paper that Janicki (1915) probably created a new genus *Foaina* which is classified in the Polymastigina, i.e., in our

definition of the *Protomonadina*, but we have been unable to obtain any references to this genus and therefore cannot say whether it is or is not classifiable in the Tetramitidae.

GENUS 17. *Macrostoma* Alexeieff 1909. The subject of this genus has already been discussed in the section dealing with the history of *C. mesnili*, and it will suffice to remind the reader that the original use of the word was *Macrostoma* Latreille 1825 for a mollusc, the second *Macrostoma* Risso 1826 for a fish, and the third *Macrostoma* Agassiz 1839 for a fish, so that Alexeieff's name was at least the fourth use of the same word for an animal.

GENUS 18. *Trimitus* Alexeieff 1910 :—This genus was created by Alexeieff for a flagellate organism which he called *T. motellae*. It is described as having two anterior flagella and one posterior which traverses the body. We have been unable to refer to Alexeieff's original paper with regard to this parasite, but da Fonseca seems to regard it as of doubtful value, and if the above are its only characters we fail to see any essential difference between it and genus 8, *Trimastix* Saville Kent 1880 and therefore temporarily, until it is further studied, exclude it from our list.

GENUS 19. *Tetratrichomonas* Parisi 1910. Alexeieff (1909) found a trichomonas-like parasite in the terminal intestine of *Salamandra maculosa*, *Triton cristatus* and *Alytes obstetricans*, and subsequently in *Haemopis sanguisuga*. This parasite, measuring 10 to 14 by 4 to 7 microns, possessed four free unequal anterior flagella and an undulating membrane thrown into long loose folds, as well as a thinnish axostyle and a nucleus rich in chromatin and bounded by a very definite membrane. He named this organism *Trichomonas prowazeki*, but as it possesses four and not three anterior unequal flagella, Parisi created a new genus with it as the type.

GENUS 20. *Chilomastix* Alexeieff 1910. Though Alexeieff started the existence of this genus in 1910 without any description, and indeed without carefully differentiating it from Tetramitus, still he rectified this in 1911, from which date the history of the genus really begins. It seems to us that the organisms to be comprised in it vary in shape but are often more or less pyriform, and that they possess three anterior flagella which are more or less equal and which spring from one, two or three anteriorly situate blepharoplasts, behind which lies the anteriorly situate nucleus. The cytostome is large and has lips possessing marginal chromatic thickenings which spring from the blepharoplasts, of which the one in the nuclear lip

is of the nature of a flagellum and can pull that side of the mouth outwards, thus forming the homologue of an undulating membrane. The other lip thickening, which is larger than the nuclear, appears to us to be morphologically a parabasal. There is no axostyle. The type species is *Chilomastix caulleryi* (Alexeieff, 1909).

GENUS 21. *Fanapepea* Prowazek 1911:—In the historical section of this paper with regard to *Chilomastix mesnili* we have a sufficient account of this genus, of which the type *Fanapepea intestinalis* Prowazek 1911 is, in our opinion, identical with *C. mesnili*.

GENUS 22. *Embadomonas* Mackinnon 1911. This genus was formed by Mackinnon for a parasite which she found in the intestine of a trichopterous larva. Her latest (1916) definition of the genus, but slightly modified, is as follows:

> 'Small, twisted, slipper-shaped flagellates characterised by a very large cytostome with prominent more or less siderophilous lips, two flagella one anterior and one cytostomic arising from two blepharoplasts situate at the anterior border of the cytostome, an anteriorly placed spherical nucleus and a definite periplast.'

As will be noted below, this definition covers the characters described by Wenyon and O'Connor in 1917 for the genus *Waskia*, which therefore becomes a synonym of Embadomonas.

The type species is *E. agilis* Mackinnon 1911, found in the intestine of trichopterous larvae and larvae of Tipula. The other known species are *E. alexeieffi* Mackinnon 1912, with prominent markedly siderophilous cytostomic lips, found in the intestine of larvae of Tipula, and *E. intestinalis* (Wenyon and O'Connor, 1917) synonym *Waskia intestinalis* Wenyon and O'Connor 1917 found in man at Alexandria.

We agree with Mackinnon that this genus is closely related to *Chilomastix*.

GENUS 23. *Protrichomonas* Alexeieff 1911. This genus was created in 1911 by Alexeieff for a parasite which he found in the oesophagus of *Box salpa*, which he called *P. legeri*, and which possessed an axostyle and three anterior flagella but no undulating membrane.

GENUS 24. *Hexamastix* Alexeieff 1912. Hexamastix was instituted by Alexeieff for a species of flagellate which he described in 1909 as being present in the terminal intestine of species of *Triton taeniatus* from the forest of Senart. This parasite resembled a Trichomastix in not possessing an undulating membrane, but differed therefrom in having six anterior flagella which were very often united in a large portion of their length. The nucleus, which contained but little chromatin, was placed anteriorly.

No name was given to this parasite, nor is there any illustration in the *Comptes Rendus de la Société de Biologie* of the Meeting of the 17th July, 1909.

Alexeieff's description resembles that of a Trichomonas found by Künstler in 1882 in the intestine of larvae of *Melolontha vulgaris*. It also had six anterior flagella and a mouth opening just behind the flagella. Bütschli placed Künstler's parasite in his new genus *Polymastix*, but he noted the difference between Grassi's and Künstler's description of the periplast.

Alexeieff (1911) again referred to this parasite, giving illustrations and pointing out that it possessed an axostyle like that of a Trichomonas and calling it *Polymastix batrachorum*.

Mackinnon (1912), in writing on *Polymastix melolonthae*, refers to this parasite in the following terms:—

> 'The flagellate with six flagella and *without striated periplast* placed provisionally by Alexeieff (1911) in this genus as *P. bufonis* seems to me a very doubtful species.'

We give this quotation in full, as it is the only reference which we possess to the name *Polymastix bufonis*.

In the same year Alexeieff instituted the new genus *Hexamastix* for the parasite known as *Polymastix batrachorum* Alexeieff 1911, of which *P. bufonis* Mackinnon 1912 must be a synonym.

The name of the parasite therefore becomes *Hexamastix batrachorum* (Alexeieff, 1911), and is at present the only species of the genus. It has an axostyle, a cytostome, and six anterior flagella, but no undulating membrane or trailing flagellum and no thickenings in its periplast.

GENUS 25. *Difämus* Gäbel, 1914:—This genus was formed for *Difämus tunensis* Gäbel 1914, a flagellate found in man and possessing all the characteristics of a species of the genus *Chilomastix* and not distinguishable from *C. mesnili*, of which it becomes a synonym.

GENUS 26. *Cyathomastix* Prowazek and Werner 1914. *Cyathomastix* owes its existence to the fact that Rodenwaldt (1911) described a human parasite which, though in general appearance like a Trichomonas, yet differed therefrom in that the undulating membrane was merely represented by an undulating peristome. This undulating margin, taking its origin from one blepharoplast, runs around the cytostomic opening and ends in the second blepharoplast. The three anterior flagella all arise from one blepharoplast along with one of the ends of the thickened cytostomic margin.

To this parasite Prowazek and Werner gave the name *Cyathomastix hominis*, and, at present, it forms the sole species of this genus. It only differs from *Chilomastix* in possessing an axostyle, and perhaps a mistake has been made owing to the line produced by the peculiar twist at times found in *C. mesnili*. At all events confirmation of its existence is required. At present we consider it to be the same as *C. mesnili*.

GENUS 27. *Hexamastix* Derrieu and Raynaud 1914:—*Hexamastix* was formed by these authors for a flagellate which they found in a case of chronic dysentery occurring in a man living at Oued-el-Alleng in Algeria. It closely resembled a Trichomonas in structure except that it possessed five anterior flagella. This parasite they named *H. ardindelteili*, but we have just noted above that the generic name *Hexamastix* has been used by Alexeieff for quite a different organism, and therefore Derrieu and Raynaud's parasite is left without a generic name, but this was altered by Chatterjee in the succeeding year in his new genus to which we will refer below.

GENUS 28. *Tetratrichomastix* Mackinnon 1914. This genus was formed by Mackinnon for Eutrichomastix-like parasites found along with *Eutrichomastix trichopterae* (Mackinnon 1910) in the alimentary canal of larvae belonging to the genus *Tipula*. The only difference between *Eutrichomastix* and *Tetratrichomastix* is that the former has three and the latter four anterior flagella.

The type species is *Tetratrichomastix parisii* Mackinnon 1914, apparently named after Parisi because he is of the opinion that the number and method of attachment of flagella are of real importance in classification.

GENUS 29. *Pentatrichomonas* Chatterjee 1915. Chatterjee instituted this genus for a flagellate which he found in the intestine of man in Bengal, and which at the time he called *P. bengalensis*. This organism agrees in most particulars except measurement with

that mentioned above under the heading *Hexamastix* Derrieu and Raynaud, and therefore Chatterjee's generic name takes the place of this Hexamastix, but Derrieu and Raynaud's specific name has priority, and the parasite becomes known as *Pentatrichomonas ardindelteili* (Derrieu and Raynaud, 1914) until some definite difference between the two is established.

More recently Chatterjee states that he has found this organism to be present in thirty-two cases of chronic dysentery.

GENUS 30. *Enteromonas* da Fonseca 1915. This genus contains one parasite, *E. hominis* da Fonseca 1915, found in the fresh faeces of a dysenteric patient in Brazil.

According to Fantham's review, *E. hominis* is a spherical or more rarely pear-shaped organism (i.e. with a tail) with a diameter varying from 5 to 6 microns, and possessing a rigid periplast which encloses an alveolar endoplasm, often with inclusions such as bacteria. In the endoplasm and lying close to the anterior end of the body is the nucleus, measuring 1 micron in diameter, and being of the nature of a protokaryon with a karyosome. Anterior to the nucleus and united thereto by a rhizoplast lies the single blepharoplast, from which three flagella arise, two of which are small and anterior while one is large and trailing.

There is no axostyle and no cytostome, while division by longitudinal fission is recorded. Da Fonseca's paper is not illustrated.

More recently da Fonseca has defined the genus and given illustrations of *E. hominis*, but this definition will be given below.

GENUS 31. *Chilomitus* da Fonseca 1915. Da Fonseca (1915) found a flagellate in the caecum in *Cavia aperea* Linnaeus 1766 (the wild ancestor of the domesticated *Cavia cobaya* Marcgrave 1648), and in *C. porcella* Linnaeus 1791 (Gmelin) which was characterised by possessing four equal anterior flagella and an anteriorly situate cytostome in close proximity to which lay the blepharoplast from which the flagella arose, and which appeared to pass out through the mouth. The blepharoplast is said to be joined to the anteriorly situate nucleus by means of a rhizoplast. There is no axostyle and no undulating membrane. The type species is *C. caviae* da Fonseca 1915.

We believe that there is no real difference between this genus and the next to be described below, viz., *Tetrachilomastix* da Fonseca 1915, because:—

1. Martin and Robertson (1912) figured the four flagella of their *Chilomastix* as escaping through the cytostomic opening in fig. 3 of their Plate 10, and also, but less markedly, in fig. 4, and partially in some other figures.

2. Da Fonseca says nothing about the siderophilous thickening of the cytostomic lips, but such a thickening is depicted in his fig. 11.

3. The general descriptions of both genera agree.

GENUS 32. *Tetrachilomastix* da Fonseca 1915. This is the name given by da Fonseca to the parasite described by Martin and Robertson (1911) as *Chilomastix gallinarum*, because it has four anterior flagella and not three.

By the kindness of Miss Robertson we have been able to examine some of the original slides of this type from the fowl, and agree with da Fonseca that it is worthy of being placed in a separate genus from Chilomastix, but we, *unfortunately*, believe that it is the same genus as that observer's *Chilomitus*, and we are not certain which name has priority, because his 1915 papers, viz., his thesis, his paper in the *Brazil Medico* of April 2nd and of September 22nd, are not available for us. In his 1916 paper he gives *Tetrachilomastix* as a sub-genus of *Chilomastix* on page 10, and *Chilomitus* as a genus on page 11.

It is therefore evident that he created *Chilomitus* as a proper genus, and *Tetrachilomastix* only as a sub-genus, but, as far as rules go, this makes no difference, and after considering matters we prefer at present to retain his name *Tetrachilomastix*, of which *Chilomitus* becomes a synonym.

GENUS 33. *Eutrichomastix* Kofoid and Swezy 1915. This is the new name proposed by Kofoid and Swezy for the genus *Trichomastix*, which name was already in use, as explained above, when suggested by Blochmann in 1884 for Bütschli's *Trichomonas lacertae*.

GENUS 34. *Waskia* Wenyon and O'Connor 1917. This genus was created in March 1917 by Wenyon and O'Connor for a small flagellate which they found in man in Alexandria, and which they named *W. intestinalis* Wenyon and O'Connor 1917.

This flagellate is oval in form, being 4 to 9 by 3 to 4 or more microns, with the anterior end rounded and the posterior end pointed, and with a cytostome at the side of the anterior end. A long thin flagellum springs from the anterior end of the body, while a second stouter and shorter flagellum arises from the inner part of the anterior wall of the cytostome, and passing backwards and outwards projects from this organella for a considerable distance. The nucleus with a central karyosome and a spherical nuclear membrane occupies the anterior end of the body. On the nuclear membrane and towards the cytostome lie two granules from which the flagella arise.

Stages of binary fission have been observed and cyst formation. The cysts are pear-shaped bodies, 4·5 to 6 microns in length.

The name Waskia was derived from the Orwa-el-Waska section of the 19th General Hospital in Alexandria.

This description must be compared with Mackinnon's definition of the genus *Embadomonas* given above.

After reading this, and comparing the illustrations, we are of the opinion that the genera are the same but the species may be different. *Waskia intestinalis* therefore becomes *Embadomonas intestinalis* (Wenyon and O'Connor 1917).

X. GENERA

After considering the various points raised above it will be observed that the following genera can be excluded from the family *Tetramitidae*, either because they belong to other families or because their names become synonyms of other genera. The genera which may be so excluded are:—

1. Chloraster; 3. Pyramimonas; 7. all the sub-genera; 10. Cimaenomonas; 13. Elvirea; 14. Costia; 17. Macrostoma; 18. Trimitus; 21. Fanapepea; 25. Difämus; 26. Cyatho-

mastix; 27. Hexamastix; 31. Chilomitus; 34. Waskia; while 12. Trichomastix disappears and becomes 33. Eutrichomastix.

After excluding the above, the family is left with nineteen genera, which may be defined as follows:—

GENUS 1. *Trichomonas* Donné 1837

Parasitic Tetramitidae possessing an axostyle, a small cytostome without thickened lips, three anterior flagella, and one posterior flagellum, which being attached to the body of the organism for a portion of its length gives rise to a well defined undulating membrane, supported by a chromatic rod or parabasal, and then ends freely. There is no contractile vacuole, and the nucleus is situate anteriorly. *Type species: T. vaginalis* Donné 1837, found in *Homo sapiens*.

GENUS 2. *Tetramitus* Perty 1852

Free living Tetramitidae without an axostyle (as far as is known) and with a large cytostome, with three anterior and one posterior free trailing flagellum, but without an undulating membrane. Contractile vacuole present. *Type species: T. descissus* Perty 1852, found in water from swamps.

GENUS 3. *Callodictyon* Carter 1865

Free living Tetramitidae with four unequal anterior flagella, and a deep ventral longitudinal furrow. *Type species: C. triciliatum* Carter 1865 synonym *Tetramitus sulcatus* Stein 1878.

GENUS 4. *Monocercomonas* Grassi 1879

Parasitic Tetramitidae with an axostyle but without a cytostome, and with four anterior flagella. *Type species: M. gryllotalpae* (Grassi 1879), from the larvae of insects.

GENUS 5. *Trimastix* Saville Kent 1880

Tetramitidae, perhaps parasitic, perhaps free living, without an axostyle (as far as is known), with one anterior, one free trailing and one cytostomic, becoming a free, posterior flagellum. A contractile vacuole is present. *Type species: T. marina* Saville Kent 1880, found in a vessel of seawater containing Fuci in an advanced state of decomposition.

GENUS 6. *Dallengeria* Saville Kent 1880

Probably parasitic Tetramitidae without known axostyle, and with one anterior and two trailing flagella, the latter arising laterally. No known contractile vacuole. *Type species: D. drysdali* Saville Kent 1880, found in animal macerations.

GENUS 7. *Polymastix* Bütschli 1884

Parasitic Tetramitidae with an axostyle, a small cytostome, four anterior flagella, and with the periplast thickened in places by ribs or folds. *Type species: P. melolonthae* Grassi 1882, found in the intestine of Melolontha, Cetonia and Tipula.

GENUS 8. *Costiopsis* Senn 1900

Ecto-parasitic Tetramitidae with dorso-ventrally flattened bodies and with a sucker-like depression on the ventral surface and an oval body, of which the true anterior end is towards the middle of the left lateral border, being marked by the origin of the flagella. Without an axostyle but with a cytostome and four flagella, two of which are long, thick and trailing, while the other two are thin and short and lie in the mouth depression or sucker. Contractile vacuoles are present. *Type species: C. necatrix* (Henneguy 1884) with numerous synonyms.

GENUS 9. *Devescovina* Foà 1905

Parasitic Tetramitidae with an axostyle, three anterior flagella and one trailing flagellum, while the thick parabasal is wound round the anterior part of the axostyle. *Type species: D. striata* Foà 1905, found in *Calotermes castaneus* Burmeister in Honolulu.

GENUS 10. *Tetratrichomonas* Parisi 1910

Parasitic Tetramitidae resembling Trichomonas but possessing four anterior flagella, two long and two short. *Type species: T. prowazeki* (Alexeieff 1909), found in Salamanders and Tritons.

GENUS 11. *Chilomastix* Alexeieff 1911

Parasitic Tetramitidae without an axostyle but with a large cytostome, the lips of which are thickened on the side of the nucleus by a flagellum which can give rise to a short membrane.

There are three anterior flagella and no contractile vacuole. *Type species: C. caulleryi* (Alexeieff 1909), found in tadpoles and in an axolotl.

GENUS 12. *Embadomonas* Mackinnon 1911

Parasitic Tetramitidae without an axostyle, but with a large cytostome with deeply staining border often thrown into folds, and one anterior and one posterior flagellum which generally lies in the cytostome. Periplast definite. *Type species: E. agilis* Mackinnon 1911, found in the intestine of larvae of Trichoptera and Tipula.

GENUS 13. *Protrichomonas* Alexeieff 1911

Parasitic Tetramitidae with an axostyle and three anterior flagella, but without a cytostome and without an undulating membrane. *Type species: P. legeri* (Alexeieff 1910), from the oesophagus of *Box salpa.*

GENUS 14. *Hexamastix* Alexeieff 1912

Parasitic Tetramitidae with an axostyle and six anterior flagella, but without an undulating membrane or trailing flagellum or thickened periplast. *Type species: T. batrachorum* (Alexeieff 1911), found in the intestine of *Triton taeniatus.*

GENUS 15. *Tetratrichomastix* Mackinnon 1914

Parasitic Tetramitidae resembling Eutrichomastix, but with four anterior flagella. *Type species: T. parisii* Mackinnon 1914, found in the intestine of the larvae of Tipula.

GENUS 16. *Pentatrichomonas* Chatterjee 1915

Parasitic Tetramitidae resembling Trichomonas, but possessing five anterior flagella. *Type species: P. ardindelteili* (Derrieu and Raynaud 1914), found in the intestine of man in Africa and India.

GENUS 17. *Enteromonas* da Fonseca 1915

Parasitic Tetramitidae without axostyle or cytostome, but with two anterior and one free trailing flagellum. *Type species: E. hominis* da Fonseca 1915, found in a case of dysentery in man in Brazil.

GENUS 18. *Tetrachilomastix* da Fonseca 1915

Parasitic Tetramitidae resembling Chilomastix, but with four anterior flagella. *Type species: T. gallinarum* (Martin and Robertson 1912), found in the caeca of fowls.

GENUS 19. *Eutrichomastix* Kofoid and Swezy 1915

Parasitic Tetramitidae with an axostyle and a cytostome, without thickened lips and with three anterior and one free trailing flagellum, but without an undulating membrane or contractile vacuole. *Type species: Eutrichomastix lacertae* (Blochmann 1884), found in the intestine of *Lacerta agilis*.

XI. AFFINITIES

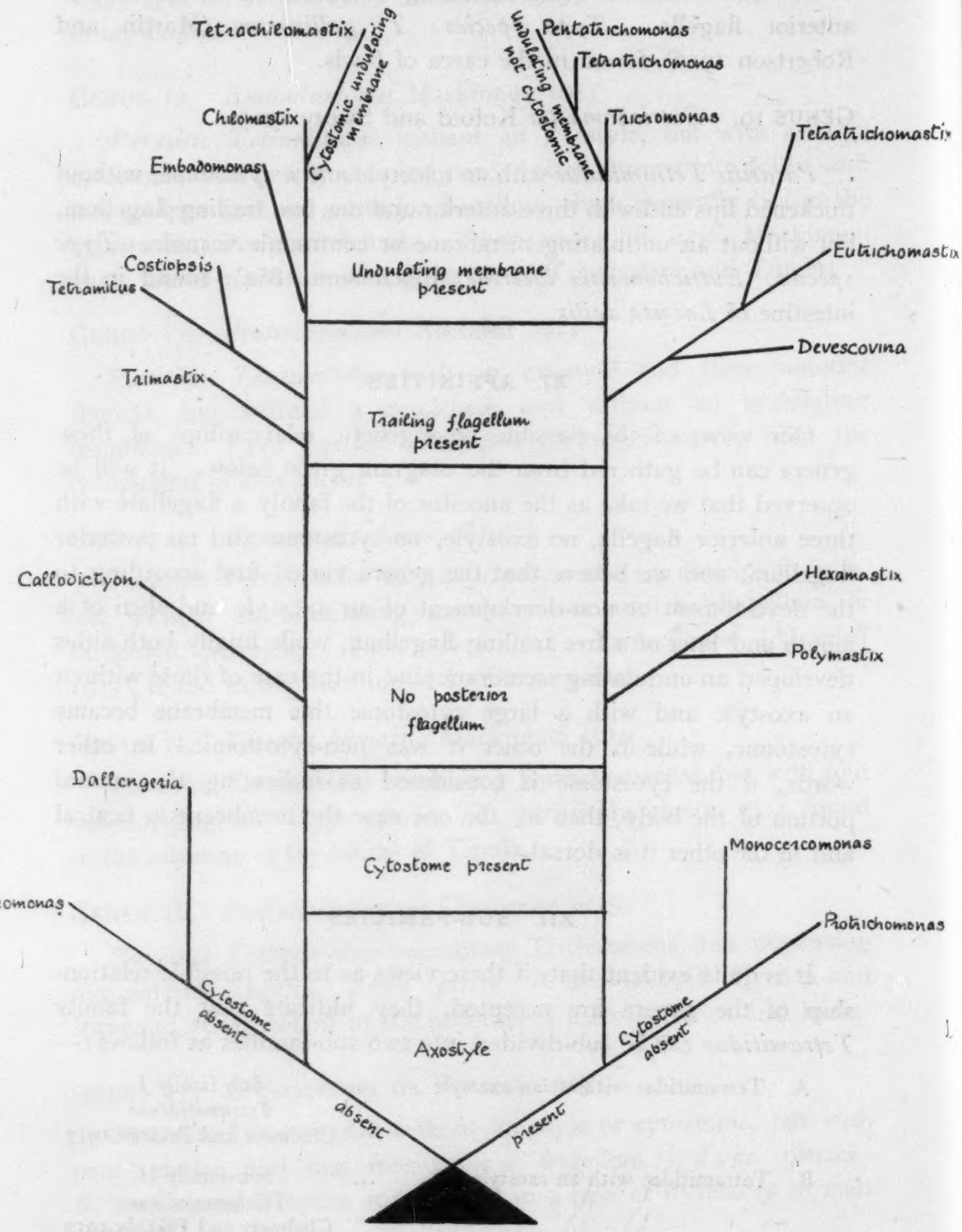
Our views of the possible phylogenetic relationships of these genera can be gathered from the diagram given below. It will be observed that we take as the ancestor of the family a flagellate with three anterior flagella, no axostyle, no cytostome and no posterior flagellum, and we believe that the genera varied first according to the development or non-development of an axostyle and then of a mouth and later of a free trailing flagellum, while finally both sides developed an undulating membrane; but in the case of those without an axostyle and with a large cytostome this membrane became cytostomic, while in the other it was non-cytostomic. In other words, if the cytostome is considered as indicating the ventral portion of the body, then in the one case the membrane is ventral and in the other it is dorsal.

XII. SUB-FAMILIES

It is quite evident that, if these views as to the possible relationship of the genera are accepted, they indicate that the family *Tetramitidae* can be sub-divided into two sub-families as follows:—

A. Tetramitidae without an axostyle	...	Sub-family I *Tetramitidinae* Chalmers and Pekkola 1917
B. Tetramitidae with an axostyle	... ...	Sub-family II *Trichomonadinae* Chalmers and Pekkola 1917

Possible Affinities of the Genera of the Tetramitidae



XIII. TETRAMITIDINAE

Further it is obvious that the parasite we are considering belongs to the Tetramitidinae, and that the genera of this sub-family are:—

1. *Tetramitus* Perty 1852.
2. *Callodictyon* Carter 1865.
3. *Trimastix* Saville Kent 1880.
4. *Dallengeria* Saville Kent 1880.
5. *Costiopsis* Senn 1900.
6. *Chilomastix* Alexeieff 1911.
7. *Embadomonas* Mackinnon 1911.
8. *Enteromonas* da Fonseca 1915.
9. *Tetrachilomastix* da Fonseca 1915.

These nine genera can be differentiated as follows:—

A. *Cytostome absent or unknown:—*

I. Two anterior and one trailing flagellum ... 1. *Enteromonas*

II. One anterior and two trailing flagella ... 2. *Dallengeria*

B. *Cytostome present:—*

I. *Trailing flagellum is free:—*

a. Body dorso-ventrally compressed and asymmetrical, ventral surface with deep depression which serves as a sucker and contains the cytostome and two short fine flagella. The two thick long trailing flagella issue from this depression ... 3. *Costiopsis*

b. Body more or less symmetrical and not compressed and arranged as above:—

1. One anterior flagellum, one said to be trailing and free, and one posteriorly directed through the body from which it escapes as a free flagellum 4. *Trimastix*
2. Flagella, three anterior and one trailing and free ... ... 5. *Tetramitus*

II. *Free trailing flagellum absent:—*

a. Chromatic thickenings in the lips of cytostome. One of these thickenings is either known or suspected to be formed by a flagellum

1. One anterior and one free posterior flagellum which generally lies in the cytostome ... ... 6. *Embadomonas*
2. Three anterior flagella ... ... 7. *Chilomastix*
3. Four anterior flagella ... ... 8. *Tetrachilomastix*

b. Chromatic thickenings not known to be present in lips of cytostome

Four anterior flagella ... ... 9. *Callodictyon*

XIV. GENUS CHILOMASTIX

As the parasite we are considering has a large cytostome, the lips of which show chromatic thickenings, one of which is a flagellum, and as it has three anterior and no free trailing flagellum, it is obvious that it belongs to the genus *Chilomastix* Alexeieff 1911.

The synonyms of *Chilomastix* are:—

1. *Cercomonas* Davaine 1880 pro parte nec Dujardin 1841.
2. *Trichomonas* Roos 1893 nec Donné 1837.
3. *Monocercomonas* Epstein 1893 nec Grassi 1879.
4. *Macrostoma* Alexeieff 1909 nec Latreille 1825.
5. *Fanapepea* Prowazek 1911.
6. *Tetramitus* Brumpt 1912 nec Perty 1852.
7. *Diſämus* Gäbel 1914.
8. *Cyaſhomastix* Prowazek and Werner 1914.

XV. CHILOMASTIX SPECIES

The species of *Chilomastix* known to us are:—

1. *C. caulleryi* (Alexeieff 1909) synonym *Macrostoma caulleryi* Alexeieff 1909, found in the intestine of tadpoles.

2. *C. mesnili* (Wenyon 1910), synonyms:—
   (*a*) *Cercomonas hominis* Davaine 1869 pro parte.
   (*b*) *Monocercomonas hominis* Epstein 1893 nec Grassi 1879.
   (*c*) *Trichomonas intestinalis* Roos 1893 pro parte nec Leuckart 1879.
   (*d*) *Macrostoma mesnili* Wenyon 1910.
   (*e*) *Fanapepea intestinalis* Prowazek 1911.
   (*f*) *Tetramitus mesnili* Brumpt 1912.
   (*g*) *Diſämus tunensis* Gäbel 1914.
   (*h*) *Cyaſhomastix hominis* Prowazek and Werner 1914.
   (*i*) *Tetramitus bocis* Brumpt 1912.
   (*k*) *Chilomastix intestinalis* Kuczynski 1914.

3. *C. motellae* Alexeieff 1912. The only reference which we possess as to this parasite is its name, which is contained in *Bulletin de l'Institut Pasteur* for 1912, page 847, and in da Fonseca's paper in 1916. It is not included in the list of species given by

Mackinnon (1916), and as we have been unable to obtain Alexeieff's original paper, we are unable to give a single *specific* character.

It seems to have been discovered in the intestine of a species of the genus *Motella* Cuvier, possibly *M. tricirrata*, but we are not certain about this.

4. *C. bocis* (Brumpt 1912) synonym *Tetramitus bocis* Brumpt 1912, found by Alexeieff in the intestine of *Box salpa* and considered to be identical with *Chilomastix mesnili*. Brumpt, however, says that Alexeieff's illustration is quite different from the human parasite, but he does not say wherein the difference lies, and therefore we retain Alexeieff's original view, that it is the same as *C. bocis*, pending further research.

5. *C. intestinalis* Kuczynski 1914. This parasite was found by Kuczynski in the caecum of *Cavia cobaya* Marcgrave 1648 in Europe, and subsequently by da Fonseca in Brazil. Its length is 13 to 16 microns and its breadth 7 to 9 microns, but as regards its structure as described or illustrated by the authors quoted, we confess to be unable to find any point of sufficient importance to indicate a specific difference between it and *C. mesnili*. The hosts of course are different, but this is not a specific character, and therefore we consider *C. intestinalis* to be a synonym of *C. mesnili*.

6. *C. bittencourti* da Fonseca 1915. This flagellate was found by da Fonseca in the caecum of *Epimys norvegicus* (Erxleben 1777) in Brazil, and we have found a similar parasite in white rats and in the gerbil (*Gerbillus pygargus* F. Cuvier 1838) in the Anglo-Egyptian Sudan.

7. *C. caprae* da Fonseca 1915. This Chilomastix was found by da Fonseca in the rumen of *Capra hircus* Linnaeus 1758 in Brazil.

8. *C. cuniculi* da Fonseca 1915. This parasite was found by da Fonseca in Brazil in the caecum of *Oryctolagus cuniculus* (Linnaeus 1758).

As a result of the above considerations we reduce the known species of Chilomastix to the following:—

1. *C. caulleryi* (Alexeieff 1909).
2. *C. mesnili* (Wenyon 1910).

XIV. GENUS CHILOMASTIX

As the parasite we are considering has a large cytostome, the lips of which show chromatic thickenings, one of which is a flagellum, and as it has three anterior and no free trailing flagellum, it is obvious that it belongs to the genus *Chilomastix* Alexeieff 1911.

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8. *Cyathomastix* Prowazek and Werner 1914.

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Mackinnon (1916), and as we have been unable to obtain Alexeieff's original paper, we are unable to give a single *specific* character.

It seems to have been discovered in the intestine of a species of the genus *Motella* Cuvier, possibly *M. tricirrata*, but we are not certain about this.

4. *C. bocis* (Brumpt 1912) synonym *Tetramitus bocis* Brumpt 1912, found by Alexeieff in the intestine of *Box salpa* and considered to be identical with *Chilomastix mesnili*. Brumpt, however, says that Alexeieff's illustration is quite different from the human parasite, but he does not say wherein the difference lies, and therefore we retain Alexeieff's original view, that it is the same as *C. bocis*, pending further research.

5. *C. intestinalis* Kuczynski 1914. This parasite was found by Kuczynski in the caecum of *Cavia cobaya* Marcgrave 1648 in Europe, and subsequently by da Fonseca in Brazil. Its length is 13 to 16 microns and its breadth 7 to 9 microns, but as regards its structure as described or illustrated by the authors quoted, we confess to be unable to find any point of sufficient importance to indicate a specific difference between it and *C. mesnili*. The hosts of course are different, but this is not a specific character, and therefore we consider *C. intestinalis* to be a synonym of *C. mesnili*.

6. *C. bittencourti* da Fonseca 1915. This flagellate was found by da Fonseca in the caecum of *Epimys norvegicus* (Erxleben 1777) in Brazil, and we have found a similar parasite in white rats and in the gerbil (*Gerbillus pygargus* F. Cuvier 1838) in the Anglo-Egyptian Sudan.

7. *C. caprae* da Fonseca 1915. This Chilomastix was found by da Fonseca in the rumen of *Capra hircus* Linnaeus 1758 in Brazil.

8. *C. cuniculi* da Fonseca 1915. This parasite was found by da Fonseca in Brazil in the caecum of *Oryctolagus cuniculus* (Linnaeus 1758).

As a result of the above considerations we reduce the known species of Chilomastix to the following:—

1. *C. caulleryi* (Alexeieff 1909).
2. *C. mesnili* (Wenyon 1910).

3. *C. motellae* Alexeieff 1912.
4. *C. bittencourti* da Fonseca 1915.
5. *C. caprae* da Fonseca 1915.
6. *C. cuniculi* da Fonseca 1915.

We give the following as a provisional diagnostic table:—

A. *Characters known to us:—*

I. *Size large; 20-25 microns in length:—*
Flagella easily seen in cysts which resemble *C. mesnili* cysts in size and appearance ... 1. *Caulleryi*

II. *Size medium; 11-18 microns in length:—*
a. Cytostome long; cysts about 7 × 5·6 microns in which the anterior flagella are difficult to see ... ... 2. *Mesnili*
b. Cytostome very short; cysts large, about 8 × 6 microns, in which the anterior flagella are very distinct ... ... 3. *Bittencourti*

III. *Size small; 7-12 microns in length:—*
a. Nucleus with a central karyosome connected to the blepharoplast by a rhizoplast; size 9-12 microns in length 4. *Caprae*
b. Nucleus without a central karyosome and without a rhizoplast; size 7-9 microns in length ... ... 5. *Cuniculi*

B. *Characters unknown to us:—*
Found in species of *Motella* ... ... 6. *Motellae*

XVI. CHILOMASTIX MESNILI

The parasite which we are considering is found in man, measures 11 to 18 microns in length, possesses a large cytostome and has cysts measuring 7 by 5·6 microns in which the anterior flagella, in contradistinction to the posterior or cytostomic flagellum and the parabasal (or siderophilous mouth parts) are difficult to see.

We therefore classify it as *Chilomastix mesnili* (Wenyon 1910), which we define as follows:—

Chilomastix of medium size, with a long cytostome and possessing cysts, measuring 7 by 5·6 microns, in which the traces of the anterior flagella are difficult to discern.

Habitat: Homo sapiens. Baboon. Cavia cobaya. Box salpa (?).

This completes our study of the classification of *this common human intestinal flagellate*, and we now turn to the consideration of its possible pathogenicity.

XVII. PATHOGENICITY

Wenyon considers that *C. mesnili* is a harmless parasite of man, and states that it can be recognised in a formed motion by its characteristic cysts. He states that it is present intermittently in the stools, but that his intermittency is not so marked as in the case of other protozoal parasites, and with this we are in agreement. We also believe that an infection can persist for years, but we believe that when the parasite increases in numbers it becomes pathogenic and causes diarrhoea.

We think that Wenyon's views and our own can be brought into agreement in that perhaps he has dealt mostly with *carriers* of the flagellate, while we have met both *carriers* and *patients*, and we use the term carrier in the same sense as when used for the *Bacillus typhosus*.

We further think that any lowered condition of the general health will enable *C. mesnili* to flourish and to cause diarrhoea, but we have never seen it cause dysentery, though, in our opinion, there is no reason why it should not do so.

It is, however, most desirable to distinguish between the pathogenicity of *C. mesnili* and that of any associated parasite, e.g. we certainly accused *C. mesnili* as being the cause of the diarrhoea which was really due to *Octomitus hominis*, which, once recognised and treated, cured the patient. On the other hand, when after prolonged search only a very few specimens of *Löschia coli* can be found and the faeces are swarming with *C. mesnili*, and when treatment for the latter parasite, which resists Emetine, is successful in stopping the diarrhoea, we think that it is the causal agent.

XVIII. TREATMENT

The treatment which we favour is to place the patient on a very restricted diet and to administer purgatives night and morning for several days consecutively, and then to give some intestinal

disinfectant, e.g. Salol. Our idea is to clear away as much of the adherent mucus of the bowel as possible and then to give whatever lethal drug is chosen by the practitioner, and we prefer Salol mixed with bicarbonate of soda. We think that we have been successful with this method of treatment.

XIX. SUMMARY

In this paper we have brought forward such facts as we have been able to observe with regard to the structure and development of *Chilomastix mesnili* (Wenyon 1910), and have endeavoured to illustrate our observations by photomicrographs.

We have also endeavoured to make clear the position of the genus *Chilomastix* as regards the other genera of the *Tetramitidae*, and in so doing have been compelled to review such information as we were able to obtain in Khartoum with regard to these various genera.

We have also attempted to differentiate from one another the known species of *Chilomastix* with the view of finding whether any animal is a carrier of the parasite. Personally, we think that man is the important carrier of *C. mesnili*, and that the infection spreads from man to man by means of the cysts, otherwise it is not possible to explain the world-wide distribution.

Finally we have discussed the pathogenicity of *C. mesnili* infections.

XX. ACKNOWLEDGMENTS

We gratefully acknowledge the kindness of Professor Stiles in lending us a copy of his valuable paper on Grassi's genera, and of Miss Robertson in sending us slides containing specimens of *Tetrachilomastix*, and the kind criticisms which Captain R. G. Archibald, D.S.O., R.A.M.C., has given us from time to time.

ADDENDUM

For the purpose of completing our remarks upon the genera of the Tetramitidae we add a diagnostic table of the sub-family *Trichomonadinae*.

DIAGNOSTIC TABLE OF THE *Trichomonadinae*

A. *Cytostome absent :—*

I. Three anterior flagella ... ... 1. *Protrichomonas*

II. Four anterior flagella... ... ... 2. *Monocercomonas*

B. *Cytostome present :—*

I. Without undulating membrane :—

a. Without trailing flagellum :—

1. Periplast thickened in places; four anterior flagella ... ... 3. *Polymastix*

2. Periplast not thickened; six anterior flagella ... ... 4. *Hexamastix*

b. With trailing flagellum :—

1. Three anterior flagella :—

x. Without parabasal ... ... 5. *Eutrichomastix*

y. With parabasal wound around the axostyle ... ... 6. *Devescovina*

2. Four anterior flagella... ... ... 7. *Tetratrichomastix*

II. With undulating membrane :—

1. Three anterior flagella ... ... 8. *Trichomonas*

2. Four anterior flagella ... ... 9. *Tetratrichomonas*

3. Five anterior flagella ... ... 10. *Pentatrichomonas*

KHARTOUM,
June 10th, 1917.

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EXPLANATION OF PLATES

PLATE VI

FIG. 1. *Chilomastix mesnili* (large form) showing the general appearance and the three anterior flagella. Note the food vacuoles and especially the large vacuole lying in the cytoplasm just internal to the mouth opening. × 3,300 diameters. Photomicrograph.

FIG. 1a. A well-differentiated nucleus showing the nuclear membrane and the karyosomes. × 2,000 diameters. Photomicrograph.

FIG. 2. The same photograph as Fig. 1, but printed lightly to show the three blepharoplasts from which the anterior flagella, the parabasal, and the posterior flagellum can be seen arising. × 3,300 diameters. Photomicrograph.

FIG. 3. *C. mesnili* showing the mouth in an open position and the posterior flagellum quiescent. × 3,300 diameters. Photomicrograph.

FIG. 4. *C. mesnili* showing the peculiar twist found at times and a widely open mouth. × 2,300 diameters. Photomicrograph.

FIG. 5. *C. mesnili* showing a widely open mouth with apparently a circumoral siderophilous band running from one of the blepharoplasts to another. × 2,000 diameters. Photomicrograph.

FIG. 6. *The Cytostome* showing the end of the parabasal on the inner lip, the posterior attachment and the outer movement of the posterior flagellum. × 3,300 diameters. Photomicrograph.

FIG. 7. *The Cytostome* to show the outward movement of the posterior flagellum and the membrane which it carries with it. × 3,300 diameters. Photomicrograph.

FIG. 8. *The Cytostome* with membrane and posterior flagellum extending so far outwards that they touch the parabasal and divide the mouth opening into anterior and posterior portions. × 3,300 diameters. Photomicrograph.



Fig. 1

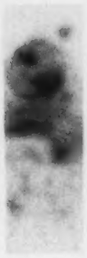


Fig. 1*a*

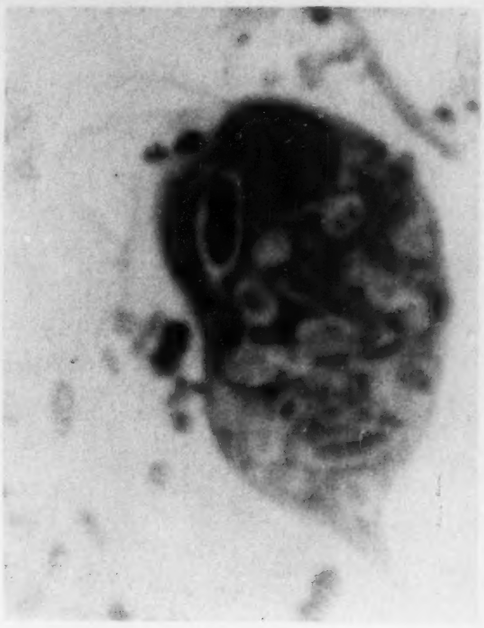


Fig. 2



Fig. 3



Fig. 4

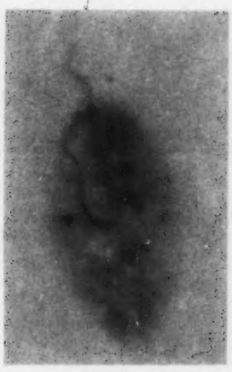


Fig. 5



Fig. 6

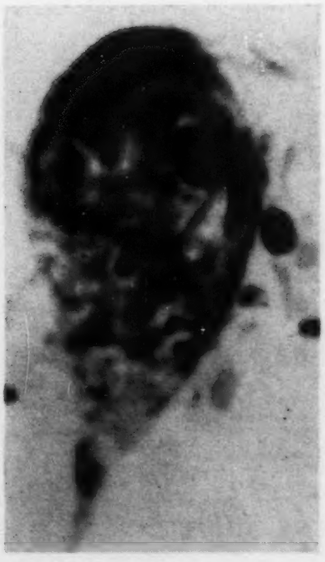


Fig. 7

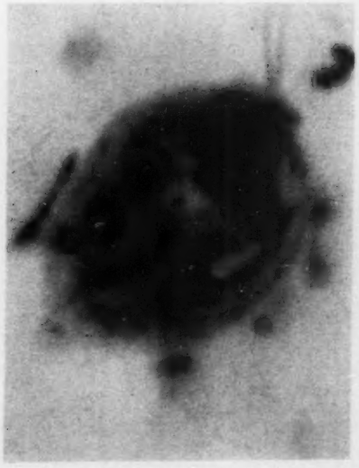


Fig. 8

Chilomastix mesnili (WENYON 1910)

Plate VII

Fig. 9. *Pear-shaped Form* showing general shape, mouth opening, with posterior flagellum and attached membrane and long caudal appendix. × 1,600 diameters. Photomicrograph.

Fig. 10. Cytostome and flagella. × 1,300 diameters. Photomicrograph.

Fig. 11. Shows slight outward movement of the posterior flagellum and its attached membrane and the general arrangement of the parabasal. × 1,300 diameters. Photomicrograph.

Fig. 12. Posterior flagellum apparently lying loose in the cytostome, probably an artefact. × 1,500 diameters. Photomicrograph.

Fig. 13. *Rounded Small Form* showing side view of mouth with apparently continuous siderophilous margins. × 1,500 diameters. Photomicrograph.

Fig. 14. Rounded Form showing the three anterior flagella. × 3,000 diameters. Photomicrograph.

Fig. 15. *Twisted Form.* × 1,600 diameters. Photomicrograph.

Fig. 16. *Tetratrichomonas gallinarum* (Martin and Robertson 1912) from a stained film kindly lent by Miss Robertson. × 2,000 diameters. Photomicrograph.

Fig. 17. *Binary Fission.* Early stage. × 2,000 diameters. Photomicrograph.

Fig. 18. *Binary Fission.* Late stage. × 1,000 diameters. Photomicrograph.

Fig. 19. *Binary Fission.* Late stage. × 1,000 diameters. Photomicrograph.

Fig. 20. *Cyst* showing nucleus, blepharoplast, posterior flagellum and parabasal. Note egg-shape and cyst wall. × 1,500 diameters. Photomicrograph.

Fig. 21. *Cyst* with clearly shown nucleus and parts of the cytostome rim. × 1,500 diameters. Photomicrograph.

Fig. 22. *Cyst* showing nucleus with large siderophilous mass lying in the cytoplasm. × 2,000 diameters. Photomicrograph.

Fig. 23. *Cyst* to show typical shape and breaking up, prior to disappearance, of the mouth parts. × 1,500 diameters. Photomicrograph.

Fig. 24. *Cyst* to show the cyst wall. × 1,500 diameters. Photomicrograph.

Fig. 25. *Cyst* showing division of nucleus. × 1,500 diameters. Photomicrograph.

Fig. 26. *Cyst* showing two nuclei somewhat obscured by overlying débris. × 2,000 diameters. Photomicrograph.

Fig. 27. *Young Form* without mouth. × 1,500 diameters. Photomicrograph.

Fig. 28. *Older Form* with mouth. × 2,000 diameters. Photomicrograph.

Fig. 29. *Larger Rounded Form.* × 1,000 diameters. Photomicrograph.



Fig. 9



Fig. 10



Fig. 11



Fig. 12

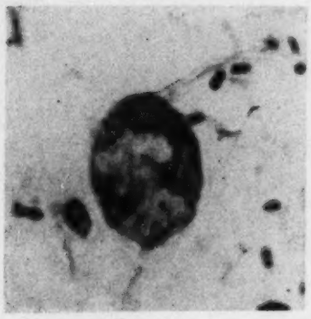


Fig. 13

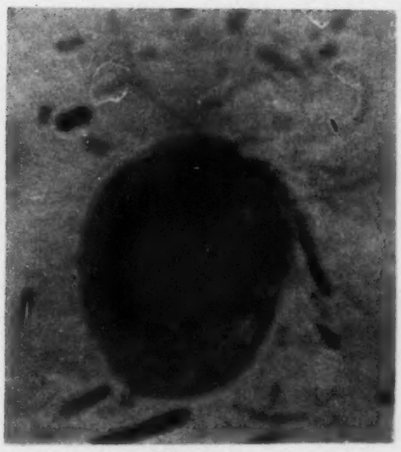


Fig. 14



Fig. 15

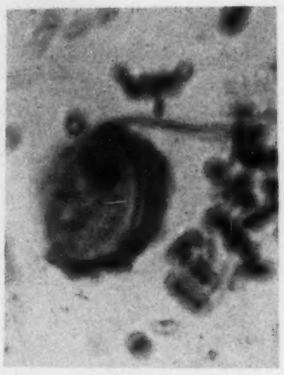


Fig. 16



Fig. 17



Fig. 18

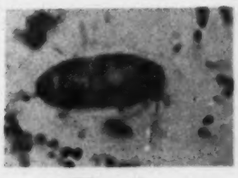


Fig. 19



Fig. 20

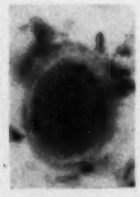


Fig. 22

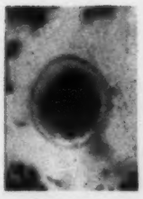


Fig. 24



Fig. 27

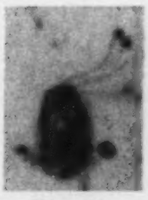


Fig. 28

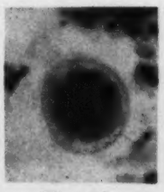


Fig. 21

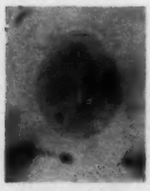


Fig. 23

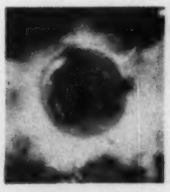


Fig. 25

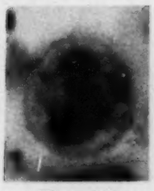


Fig. 26

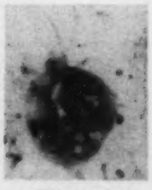


Fig. 29

Chilomastix mesnili (WENYON 1910)

NOCARDIA CYLINDRACEA: A SOUTH AFRICAN OTOMYCOSIS

BY

W. E. DE KORTÉ, M.B., Lond.

(*Received for publication* 18 *July*, 1917)

Patient, man aged 50, ascribes the trouble to a prick on posterior surface of ear with forage, bales of which he was helping natives to lift on to a wagon. The whole of the ear is greatly swollen and distorted, and brawny swelling of a dusky red colour extends for about two inches around the insertion of the ear. Several small sinus-like openings occur on the external surface of the ear, from which a thin yellowish oily fluid exudes containing small flocculi. This discharge smells strongly of old boot leather. After three weeks of the administration of 45 grains of iodide of potash per diem nearly all the germs which had previously no relationship to the polymorphonuclear leucocytes were now found within these cells. After six months' treatment with the above dose of iodide of potash the brawny swelling was contracting in extent, and over the mastoid was so hard to the touch as to give the idea that the bone was affected. On prolonged firm pressure pitting could be noticed. As the condition appeared stationary, the iodide was increased to 90 grains a day without any sign of iodism. On squeezing the swollen pinna between the fingers distinct crepitation could be heard. The patient states that the affected area seems to swell up suddenly, then after a variable period of tension to break down, an increased quantity of fluid escaping from the various openings. The only visible effect of the iodide of potash was that it localised the mischief. It was next resolved to try the effect of a vaccine made from the acid-fast oidial. A ten days' growth on a glucose agar tube was pulverised and suspended in 10 c.c. of water and 0·5 c.c. injected. This caused considerable local reaction with great redness and swelling of the skin lasting for six days round the site

of inoculation. After three such injections, the original incision wounds made under the impression that the condition was one of ordinary suppuration and the various sinus openings—which up to now had shown no disposition to heal and which had been discharging for three years—began to granulate, and the brawny swelling over the mastoid broke down into a deep punched-out ulcer. Two more doses of vaccine were given, and as the injections seemed to exhaust the patient the iodide was stopped. The dose of vaccine was now increased to 0·75 c.c., and this caused a very violent general reaction which prevented sleep for two days. After another dose of 0·75 c.c. of vaccine, all the lesions, with the exception of a long narrow crack which extends from the level of the fossa of the antehelix almost to the free edge of the lobule and which has only appeared recently, are healing. As the patient seemed much weakened the inoculations were stopped, and the ear simply washed with peroxide of hydrogen. A hole has made its appearance in the upper portion of the punched-out ulcer at the back of the ear, through which fluid can be syringed into the meatus. A bursal-like swelling has appeared at the back of the ear at its junction with the scalp at the level of the antitragus. The ear is now much smaller in size and almost natural in shape, though the margin of the helix looks as though pieces had been gnawed out of it. Sixty days after the first inoculation several of the injection sites on the abdomen which had remained as hard lumps began to break down and discharge a thin watery fluid, and on the one hundredth day the site of the first inoculation similarly broke down. There can be little doubt that the effect of the inoculations was to cause necrosis at the foci of intensest infection.

All observation was now suspended, as the patient left for his distant home.

Two elements besides ordinary bacteria are seen on examining a smear of the discharge. The one an acid-fast bacillus (fig. 1) 2 to 6μ in length, the other a non-acid-fast spindle-shaped element 8 to 12 μ long and 1 to 5 μ in diameter. The latter occur free or are collected in spherical masses in a faintly staining matrix. These are considered to be grains (fig. 2), except that they are microscopic,

measuring from 24 to 30μ in diameter, and seem analogous to the grains of other mycetoma. Although the spindle-shaped element occurs for the most part singly, some long threads, 140μ or over, which are a succession of spindles, are occasionally seen. After four injections of vaccine (*vide* clinical history) convoluted mycelial threads were found in the grain. The grain is often surrounded by a palisade of two or three layers of a cell with faintly staining nucleus and cytoplasm, unlike the usual plasma cell and more like a small myelocyte. No true branching occurs in either the acid-fast or in the spindle-shaped element, nor does either element contain spores, though true branching, as well as spores, occur on culture. Perhaps the latter reference to spore formation needs some qualification, for after the patient had been taking 45 grains of potassium iodide per diem for fourteen days deeply staining refractile dots resembling spores made their appearance, both at the extremities and in the course of the acid-fast rod, and the protoplasm generally did not stain so uniformly as before. (fig. 3. × 1500 dia.).

Aërobic cultures of the pus from the ear on agar failing, glucose agar shake-cultures were made. In ten days a few (two to three) delicate almost transparent lamellae made their appearance in the depth of the medium. These lamellae are denser at one edge and shade off into invisibility at the opposite edge; they are somewhat curved on themselves and resemble a lady's Spanish back-hair comb, except that they are not serrated like the comb. In two of the three lamellae present in the culture the tendency to curve was carried to the extent of forming a perfect cylinder, and it is this appearance which suggested the name of *cylindracea*. If such a small cylinder is viewed on edge from its denser margin it resembles almost exactly a ring of tobacco smoke in shape and translucency. The diameter of such a cylinder could not be determined, but magnified 6 diameters it appeared to be about 1 mm. Owing to the great difficulty of getting these elements in pure culture and the consequent fear of losing them, no microscopic examination of them was made. Sub-cultures were made from one of these cylinders in six vaseline glucose broth tubes, by which is meant glucose broth covered with a layer of vaseline and then sterilized. In one tube only did growth occur. The appearance of this growth after twelve weeks' incubation at 37° C. was that of a hollow sphere; earlier in its development

there appeared to be a condensed ring from which growth proceeded. The semi-transparent spheroidal growth rested at the bottom of the tube and the medium remained quite clear. The growth was tough, and some force was necessary to detach a piece of it. Under the microscope this is seen to consist of a thick felt work of branching filaments, a very few of which show a small bulbous enlargement at the free end (fig. 4).

All the filaments are gram-negative, non-acid fast, and vary considerably in thickness; the protoplasm in some lengths stains evenly, while in other portions the stain is not so evenly distributed. No clubs are to be seen, no definite septa are recognisable and no free spores occur, though the bulbous enlargement above referred to as occurring on some hyphae may be spores. The appearance of such a mycelium is very similar to that of actinomycosis hominis, and is quite unlike the spindle-shaped hypha present in the discharge from the affected ear, in which, as before remarked, no true branching takes place. Though repeatedly attempted, no sub-culture of this artificial mycelium could be obtained on vaseline broth, though at the end of nine months' incubation a faint, white opacity was observed in the upper part of the broth medium, which disappeared on shaking. Microscopically the opacity consists of an amorphous detritus; sub-cultures of this detritus were made on the surface of ordinary agar, as a shake-culture in glucose agar and on blood-smear agar. No development took place on any of these media even after months. It would appear therefore that the branching non-acid-fast mycelium is not only an artificial stage in the life of the organism, but also one that could not reproduce itself as such. It undergoes morphosis if placed in a suitable environment, or degeneration in an unfavourable one.

Some of the original mycelial growth was next inoculated on a blood-smear agar tube to which some sterile cerebro-spinal fluid was added, and incubated aërobically. At the end of sixty days' incubation—precaution having been taken to prevent evaporation of the fluid—half a dozen or so pale lemon-coloured colonies were found adherent to the surface of the agar, so that only a complete colony could be detached (fig. 7). Such a colony was with difficulty broken up and spread as a uniform smear on a slide, and consists entirely of a bacilliform, which is acid fast to 20 per cent. of sulphuric acid.

These acid-fast elements, resembling in appearance tubercle bacilli, vary considerably from 1 to 4μ in length and 0·3 to 0·5μ in thickness. There is also some variation in shape, some being rod-like, some sausage-shaped, others comma-like, and nearly all slightly curved. The protoplasm stains unevenly, and it is difficult to decide whether points of more intense staining, which occur terminally and in the course of the rod, are spores or merely beading such as the tubercle bacilli presents (fig. 5). No definite clubs are met in such a culture, but some of the elements are wedge-shaped. All the elements of this primary culture are gram-negative, but in sub-cultures on glucose agar, rods occur presenting dots which are gram-positive, while the rest of the contents is gram-negative. It was now felt that as the acid-fast bacilliform had been seen to be descended from the artificial non-acid-fast mycelium, it would be of interest to re-convert the former into the latter. With this object some of the primary acid-fast culture was inoculated into a vaseline broth tube, but even after months no growth occurred. The explanation of this failure is probably the anaërobic form of culture and the absence of glucose, for, if some of the acid-fast element is grown on an agar glucose shake culture a plentiful growth occurs on the surface of the agar, but not in its depths; such a sub-culture does not differ from the original one. To avoid confusion and repetition in the subsequent narration of the life-history of the organism, it is proposed to apply the term 'oidial' to the acid-fast bacilliform hypha derived from the mycelium, 'sporoidial' to its descendants, and the term 'arthroidial' to the non-acid-fast spore-bearing hypha and its descendants; the term 'mycelium' being reserved for branching hyphae. Sub-cultures of the oidial were also made on glucose agar smeared with blood and to which cerebro-spinal fluid was added. On this medium there was a good growth. Aërobic and anaërobic cultures of the oidial were made in sterile ovarian fluid, but in the absence of oxygen there was no growth, though on the fifty-eighth day a few of the oidial forms still persisted in the odourless fluid. In the aërobic culture on the forty-sixth day the fluid had a penetrating smell of old boot leather or of a tannery. This smell was the same as the odour of the patient's ear. Under the microscope relatively few bacilliform elements were to be seen; such as were present varied in length, and almost all, with the exception of a

few, had lost their acid-fast property. In addition to these non-acid-fast bacilliform elements, great numbers of circular thin-walled spores had made their appearance. The morphology and acid-fast property of the oidial being determined by sub-culture to be constant for a glucose agar environment, a similar experiment was made with the sporoidial, and this, too, was found fairly constant on glucose agar, but not so markedly as the oidial itself, variations in the relative length of the rods and proportion of rods to spores, as well as in their staining reactions, being found. It was now fairly apparent, too, that the various stages of the parasite could be perpetuated more or less by a constant environment; it was also found that a change of phase was determined by the progressive or retrogressive state obtaining in the parasite itself, and that such a state was related to the age of the culture at the time of sub-culturing. Originally, as before remarked, all sub-cultures from the mycelium failed on such media as were tried, excepting that on the cerebro-spinal fluid-blood agar medium, but, a glucose agar shake-culture being made from a seven months old culture of the mycelium—which was now almost dissolved in the broth, and from having been an upstanding spheroid, had by this time degenerated into a flaccid, almost transparent, jelly-like residium—no growth took place in the depth of the agar, but on its surface a dirty white pellicular growth occurred in six days' time. This was found to consist of a gram-negative non-acid-fast bacilliform hypha and a large number of thick-walled spores, some of which were free (fig. 6), while some occurred in the course of the rods or were terminal. Various modes of spore staining were tried without success, as these spores were immediately decolourised by acids or alcohol, differing in this respect from the sporoidial spores which retained their acid-fastness. A sub-culture of this arthroidial was made on blood agar, and by the fortieth day the haemoglobin in the upper part of the medium was completely digested, though a slight red tinge still remained in the cylindrical portion of the agar. Some of the growth had collected as yellow oily balls in the water of condensation. A scraping from the surface of the blood agar consists of a mass of very faintly staining degenerate bacilliform hyphae; very few spores and no acid-fast elements are present. The growth in the water of condensation consists of a feltwork of vaguely staining degenerate threads, among

which are to be seen a number of definite branching threads rich in staining substance which tend to break up into bacillary forms; a few spores are to be seen, and some acid-fast dots are contained in what appears to be a swollen and degenerate hypha. The same culture as above examined on the twenty-sixth day and stained as before with carbol-fuschin and methylene blue showed the same appearances, but the readily staining threads were not so numerous and tended more to breaking up into bacillary forms. While it is manifest that the sporoidial elements are more or less identical with those described above as arthroidial, there are minor microscopic differences between them: thus the bacilliform hyphae of the former are more uniform in length and breadth and stain better than do their arthroidial homologues; the spores derived from the oidial, too, are more circular, smaller, vary more in size from splitter to shaped spores and have not such thick walls, while a larger proportion of them are acid-fast than is the case with the arthroidial spores, which are more constant in size and shape, are generally oval and measure 2μ. The question naturally arises, what is the sequence of the development of the non-acid-fast hyphae and spores from the acid-fast oidial? Does the latter break up into spores first, which in turn give rise to the non-acid-fast hyphae, or does the oidial first lose its acid-fastness, change into non-acid-fast hyphae which beget spores? From the fact that rods are seen bearing terminal spores which may be either acid or non-acid-fast, but no interstitial spores, and that the size of the acid-fast particles vary from splitter to recognisable spores, it seems probable that the acid-fast cytoplasm breaks up into spores first and that these latter give rise to the non-acid-fast hypha. On the other hand, with the arthroidial the appearances suggest that the spore grows at the expense of the bacillus-like hypha, absorbing its substance as it develops, and that each hypha gives rise to one spore only.

CULTURAL PHASES OF *NOCARDIA CYLINDRACEA*

Original glucose agar shake-culture from pus 10-14 days' incubation: comb-like lamina becoming a cylinder; character of growth not determined

In vaseline broth after 40 days' incubation: spheroidial growth consisting of branching, gram-negative, non-acid-fast mycelium resembling in appearance actinomycosis hominis: when 60 days old transferred to :—

Glucose agar shake and surface cultures: sterile

When 220 days old transferred to :—

Vaseline broth: After 12 months' incubation, white powdery appearance in upper part of broth, amorphous growth

- Blood agar: sterile
- Glucose agar: sterile

Blood agar plus cerebro-spinal fluid: after 60 days' aërobic incubation acid-fast bacillus resembling T.B., forming discrete, heaped-up colonies like earth worm casts—the oidial

Glucose agar, aërobic, constant acid-fast bacilliform growth

- Aërobic, ovarian fluid: 45 days' incubation, stench of tannery, jelly-like growth some of which floats, consisting of spore bearing mostly non-acid-fast spores and hyphae—the sporoidial
- Anaërobic, ovarian fluid: sterile

Glucose agar aërobic culture: 10 days' incubation slimy, yellowish growth of non-acid-fast spore bearing hyphae—the arthroidial

Glucose blood agar: digestion of haemoglobin, growth consists of spore bearing non-acid-fast hyphae which form oily balls in H_2O of condensation

	OIDIAL	ARTHROIDIAL
Glycerine-Potato ...	No growth	Thin, scanty, dirty yellow growth discolouring fluid in bulb of tube as well as potato itself.
Gelatin, mean 22° C.	No growth or liquefaction, three weeks or longer.	No growth or liquefaction, three weeks or longer.
Glucose Agar ...	Shake culture: No growth in depth of agar, only on surface. Surface culture: Solid, heaped-up, discrete, very pale yellow growth which tends to spread on to the walls of the tube.	A profuse, dirty yellow slimy growth.
Blood Agar	Solid, heaped-up, discrete, pale orange growth. No digestion of haemoglobin.	A dirty yellow, slimy growth which collects in water of condensation as oily balls. Haemoglobin digested.
Litmus-Milk	16 days' incubation: colour of litmus slightly dissipated, precipitation of curd as fine flocculus which is slightly peptonised.	Colour of litmus entirely dissipated, precipitation of curd as fine, fawn-coloured flocculus and more markedly peptonised.
Inspissated Sheep Serum.	20 days' incubation: slight digestion of stratum with formation of perfectly clear fluid with thin scum floating on it.	Digestion of stratum more marked with formation of opalescent, fawn-coloured fluid with slight dirty yellow scum on its surface.
Ovarian Fluid ...	Anaërobic culture: sterile. Aërobic culture: intense smell of tannery, a greyish, tough, slimy growth, part of which floats. On this medium the oidial is converted into the sporoidial, which is practically identical with the arthroidial.	
Vaseline Broth ...	No growth.	

GENERAL CONCLUSIONS

1. That the branching mycelium is an artificial product, and that it cannot reproduce itself as such.

2. That the occurrence of large numbers of comparatively thick-walled arthrospores in conjunction with bacilliform hyphae is also artificial.

3. That the occurrence of the artificial branching mycelium and bacilliform hyphae bearing spores may be heterœcious phases in the life cycle of *N. cylindracea* which occur in another host—forage.

4. That the adoption in this instance of acid-fastness in a bacillary form is probably an aid to pathogenicity. *Vide* clinical history.

5. That apparently the organism is anaërobic in certain phases and aërobic in others.

6. That the only form of reproduction in man appears to be by spores situated on hyphae.

ACKNOWLEDGMENT

I desire to thank Dr. James Luckhoff for his great kindness in placing the case at my entire disposal for the purpose of investigation.

EXPLANATION OF PLATES

PLATE VIII

Most of these illustrations may, with advantage, be examined by means of a reading lens.

Fig. 1. *N. cylindracea.* Acid-fast element in pus from ear. × 700 diameters. Photomicrograph.

Fig. 2. *N. cylindracea.* The grain showing spindle-shaped non-acid-fast hyphae in faintly staining matrix, from pus of ear. × 700 diameters. Photomicrograph.

Fig. 3. *N. cylindracea.* The acid-fast element showing spores (after fourteen days' treatment with iodide of potash). × 1,500 diameters. Photomicrograph.

Fig. 4. *N. cylindracea.* The artificial branching mycelium from a culture in vaseline broth, showing a small occasional bulbous enlargement on a few of the hyphae. × 700 diameters. Photomicrograph.

PLATE VIII

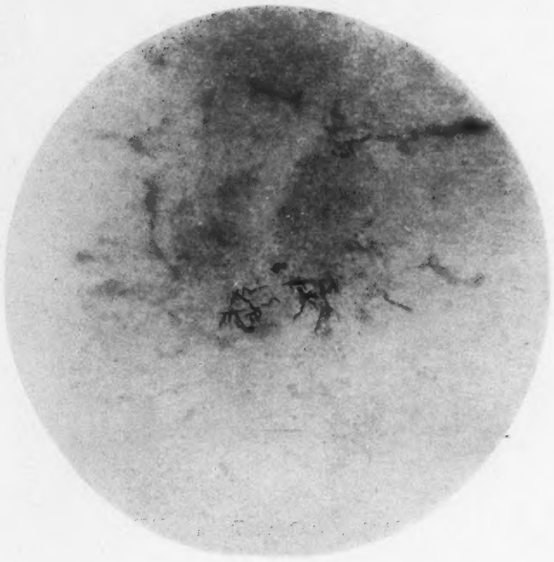


Fig. 1

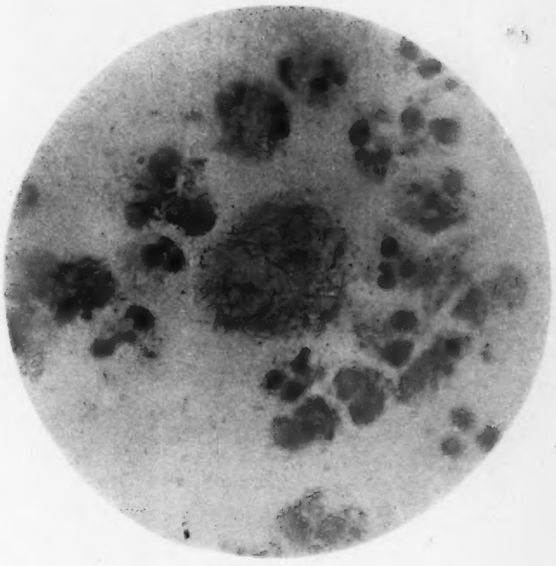


Fig. 2

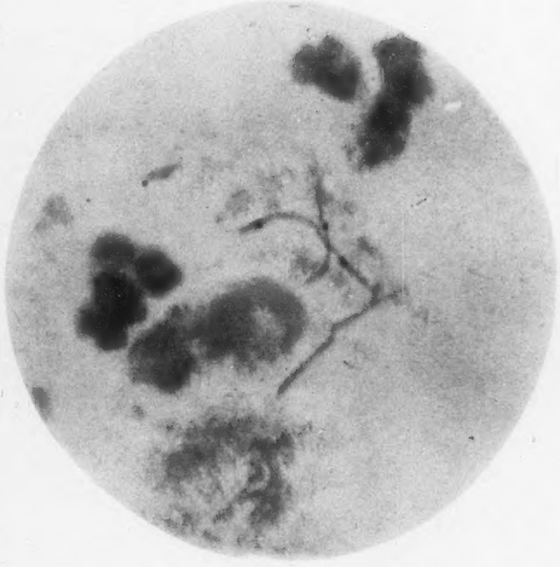


Fig. 3

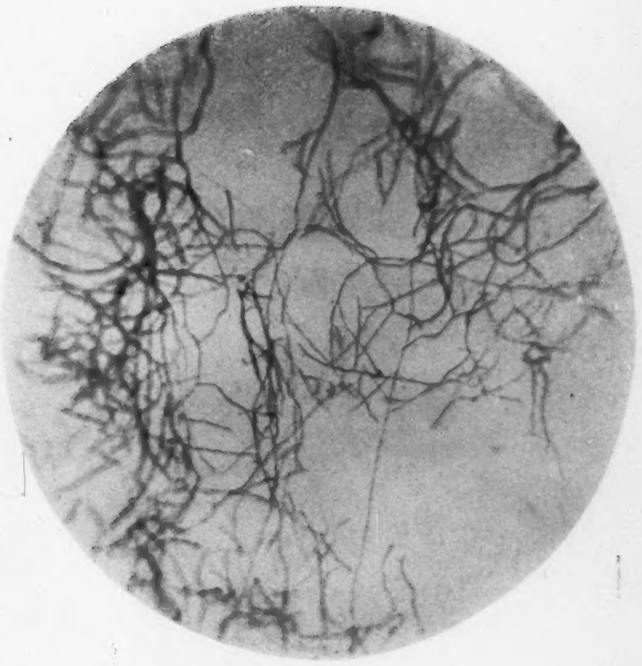


Fig. 4

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PLATE IX

Fig. 5. *N. cylindracea.* Culture of oidial on glucose agar. × 700 diameters. Photomicrograph.

Fig. 6. *N. cylindracea.* Culture of arthroidial on glucose agar showing bacilliform hyphae and spores. × 700 diameters. Photomicrograph.

Fig. 7. *N. cylindracea.* Culture of acid-fast oidial on glucose agar thirty days old, showing discrete colonies and tendency of growth to spread on walls of glass tube. Photograph.

Fig. 8. *N. cylindracea.* The affected ear. Photograph.

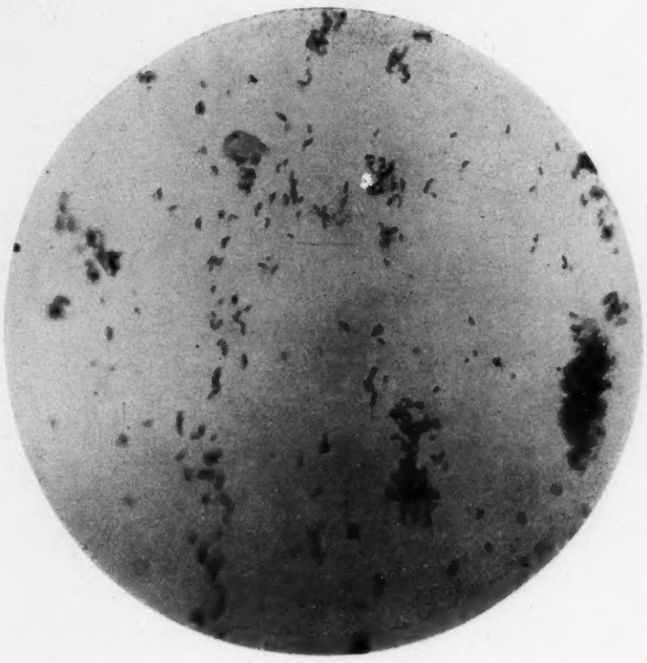


Fig. 5

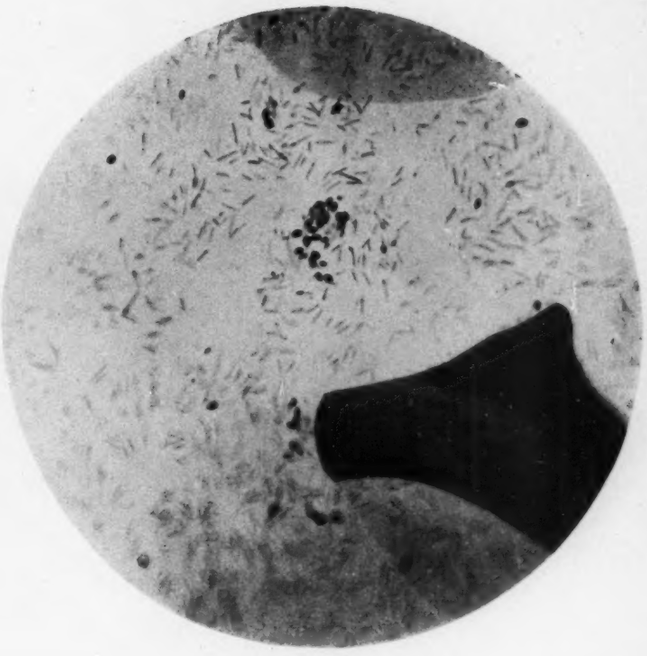


Fig. 6

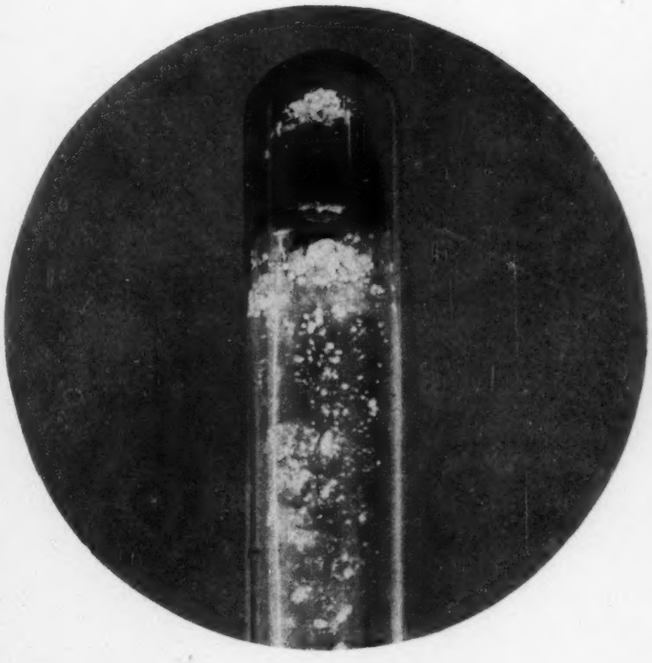


Fig. 7



Fig. 8

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TSETSE FLIES AND FLY-BELTS

BY

CUTHBERT CHRISTY, MAJOR R.A.M.C.

(*Received for publication* 26 *November*, 1917)

A 'fly-belt' is not a region over which 'fly,' that is, species of *Glossina* belonging to the *morsitans* group, is always to be found, but a district beyond which the fly is rarely to be met with. The fly is not uniformly distributed over this area. One may pass a belt and see not a single fly, yet a month afterwards one is tormented by the pest.

It is common knowledge that the fly migrates *en masse* from one part of the belt to another. No one seems yet to have afforded a satisfactory explanation why it should always migrate within certain limits. The country beyond the fly-belt is often identical in character and type of vegetation, so far as one can see, to that within it. Where a road passes through a belt, flies that have followed animals or men beyond the confines of that belt invariably fly back to it on giving up the chase. At least, if one returns along that road soon after they have left, not a fly is to be encountered until reaching the belt again.

Beyond these belts there is something inimical to the fly, some enemy known to the fly but not to us. There are instances, for example, in connection with the rinder-pest epidemic which swept over part of Africa about 1896, where the enemy seems to have raided the fly-belt and gained the upper hand, causing the fly to disappear from the district never to return, but whether owing to death or migration is uncertain.

Within the belt, the fly has two forms of migration. One it is easy to explain. It is the annual movement, due to the burning of the bush in the dry season, to the streams, khors, wadis and moist places, where shade may be found and shelter obtained from the fire and smoke. For two or three months each year all the flies in a fly-belt are to be found in such places and not in the burnt bush. To enter

or pass through these sanctuaries after 10 a.m. during this period is like fighting a swarm of fussy, angry bees. In the early morning one passes them with comparative impunity if noise and disturbance are avoided.

Not until the first spring showers arrive and the burnt and blackened bush begins to take on its normal green appearance again do the flies leave their shelter for the open country.

The second form is that which goes on throughout the rest of the year, and is far more difficult to understand. One month the flies are there, but the next they are gone to some other part of the belt. So much we know, but what is not yet certain, is whether the fly has definite months of migration, that is, whether its local migrations take place at the same time each year or month, and what prompts them.

I am convinced that animals play a small or no part in the mystery. I have been 'eaten up' by fly when after buffalo, yet a week later have found the same herd in the same place but not a fly with them; and *vice versâ*, I have found buffalo amongst swarms of fly, put them on the move, tracked them for miles, and come up with them, still in the fly-belt, without a single fly in attendance so far as I could see. As a rule, buffalo move from one district to another at night or early morning when the fly cannot follow them. On several occasions I have found areas of country swarming with fly, but obviously with extremely few animals in it, large or small. The most conspicuous feature of such areas in the Upper Bahr-el-Ghazal is tall spear-grass.

In some districts in the Eastern Welle basin and in the Bahr-el-Ghazal, *Glossina morsitans* is to be found in millions. Some of these districts are inhabited, some absolutely without inhabitants for many days' march; while in some game is very scarce, in others animals are to be seen in the open areas in hundreds. The presence or absence of game depends upon grazing facilities, but these have no relation to the presence or absence of fly, nor has the prevalence of sleeping sickness any relation to the number of flies (*morsitans*), or to the number of game in any given area, or even to the presence of game at all, so far as these districts are concerned.

That wild animals may be a reservoir for the trypanosome of sleeping sickness is not unlikely, but to assume that they are the chief

reservoir of the disease, because a trypanosome sometimes found in them cannot be distinguished microscopically from the trypanosome known to cause the disease in man, seems to me, knowing Central Africa and the natural history of its animals as I do, a most dangerous assumption. Even if they were proved to be the chief reservoir, no one who knows his Tropical Africa could ever suggest in earnest the possibility of exterminating all wild animals in any part of it, except perhaps a desert, or the probability of the extirpation of the disease if they were all exterminated.

If in speaking of wild animals or great game the antelopes are referred to, then I personally am convinced that they play a quite negligible part, if any, in the transmission of the disease to man. If, however, the subject is approached more cautiously and the life-history of each animal is studied, together with the daily life of the African native in a sleeping sickness area, it is possible to exclude almost with certainty most of the wild animals, and to point the finger of suspicion to just one or two. Of these the pig, in my opinion, will be found to be the chief culprit. Not only the common red river-hog and the wart-hog, but more especially the semi-domesticated pig frequently seen round about native villages.

In the reports of the Liverpool School of Tropical Medicine Sleeping Sickness Expedition to the Congo, so far back as 1904, the possibility of pigs being implicated in the transmission is mentioned. On several occasions, whilst travelling through the Cataract Region of the Lower Congo, Dr. Dutton and myself, members of that Commission, had opportunities of noting that *Glossina palpalis* was frequently to be seen in the ears of the pigs, and was in that way carried considerable distances from the water. Since then the indictment against the pig has been strengthened from time to time, and very recently by additional work carried out in the Belgian Congo.

During my last journey in the Bahr-el-Ghazal and Congo, I attempted to collect evidence for or against the theory, that wild animals were an important reservoir for sleeping sickness, by making as soon after death as possible a microscopic examination of the blood of each animal shot.

From two to six fresh blood specimens were taken from each animal.

Out of 160 animals, giraffe, elephant, buffalo, duiker, pig, colobus monkey, etc., only five were found to have trypanosomes in their blood, and only one, a wart-hog, out of all the number, had a species of trypanosome which, if it were not, could be mistaken for the trypanosome of the disease in man. Three of the others were infected with a short, active trypanosome of the *pecorum* type, while in the fifth, one long thin trypanosome was observed.

The number of animals infected may in reality have been slightly higher, for it frequently happened that the slides could not be examined for some hours.

Dogs and monkeys were obtained for inoculation, but, owing to the impossibility of personally watching these after inoculation, results obtained from this source were discarded.

Other animals found infected were the waterbuck and bushbuck. Those most frequently examined were Jackson's hartebeeste and the Kobs (*Cobus thomasi* and *Cobus vaughni*). Special care was exercised in taking and examining the many buffalo slides, but on no occasion were trypanosomes found in any of them.

November 4th, 1917.

REFERENCE

DUTTON, TODD and CHRISTY (1904). *Liverpool Sch. Trop. Med.* Memoir XIII.

STUDIES IN THE TREATMENT OF MALARIA

VI. ORAL ADMINISTRATION OF QUININE FOR TWO CONSECUTIVE DAYS ONLY IN SIMPLE TERTIAN MALARIA

BY

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Undertaken at the request of the War Office

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We have shown in a previous paper (1917) that in simple tertian malaria the intramuscular injection of grains 15 of quinine bihydrochloride on two consecutive days controls the febrile paroxysms and causes the disappearance of all stages of the parasite from the cutaneous blood. We then proceeded to ascertain what would be the effect of the same dose of quinine sulphate given by the mouth on two consecutive days, and further, in order to ascertain the effect of the magnitude of the dose, a series of observations was made with the following doses given on two consecutive days:—5, 10, 30, 60 and 90 grains. So far as possible, such cases as did not relapse were observed for a period of at least 60 days after treatment in order to determine the curative* value of the respective treatments.

*By 'cure' we mean no relapse during an observation period of at least two months after cessation of treatment.

All the cases, except some of those in the Grains 90 series, were adult males infected in Macedonia at least nine months previously, and all had had more or less quinine during this period. In the Grains 90 series five cases were infected in other countries (Mesopotamia, France and India; *vide* Table VIII).

Owing to the large number of cases considered in this paper, it is impossible to reproduce the temperature charts of every case. A few only are given illustrating the usual course of the temperature curve, or some noteworthy departure therefrom, after each of the various treatments.

In the temperature charts and tables:—

Q.O. = oral administration of solution of quinine.
gr. = grains of quinine sulphate or bihydrochloride.
T. = simple tertian trophozoites or schizonts.
G. = simple tertian gametes.
t. = malignant tertian trophozoites.
cr. = malignant tertian gametes.
Neg. = no parasites found.

5-GRAIN (QUININE SULPHATE) SERIES (Cases 121-132)

Quinine sulphate in solution was given in 5 grain doses on each of two consecutive days in twelve cases. The results are recorded in Table I.

In five of the twelve cases parasites disappeared from the blood in one to two days, in three cases in three days, and in four cases they did not disappear.

In one case the temperature fell to normal on the day of the first dose, in nine cases in one to three days, in one case (No. 128) in eight days; in the remaining case (No. 127) there was continuous low intermittent fever for fifteen days, which subsequently subsided, there being no relapse for an observation period of sixty days.

Relapses. In four cases there was no effect on the parasites; in seven of the remaining eight cases parasitic relapses occurred in five to eighteen days; in the remaining one (Case 127) there was no parasitic relapse in sixty days. In ten cases febrile relapses occurred in eight to fifteen days after the first dose; in one instance (Case 129) no relapse occurred in eighteen days when the patient left hospital; in the remaining case (No. 127), as stated above, treatment did not control the temperature.

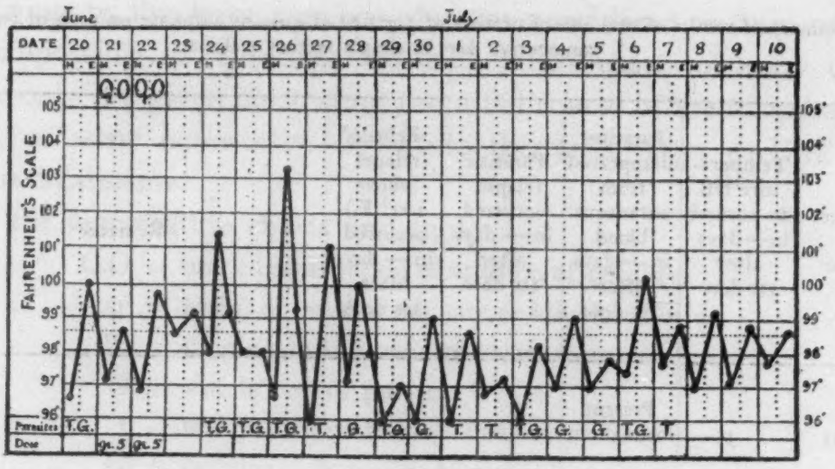
TABLE I.

Summary of results of oral administration of 5 grains of quinine sulphate on each of two consecutive days in simple tertian malaria.

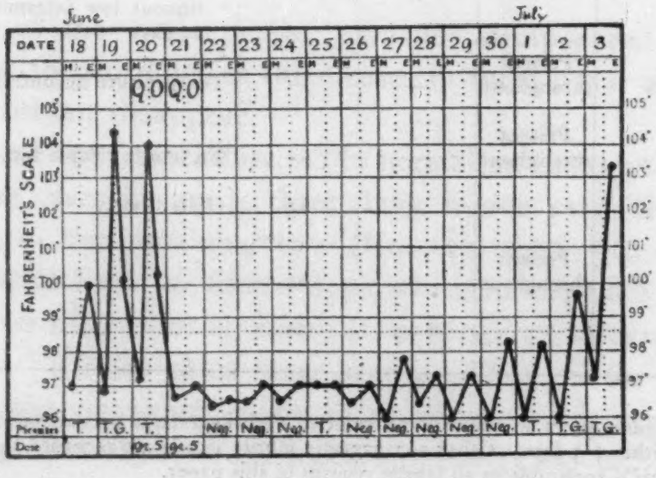
Number of Case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Parasitic relapse occurred in — days after 1st dose	Febrile* relapse (above 100° F.) occurred in — days after 1st dose	Remarks
121	3	Present throughout	—	8	
122	Same day	1	18	10	
123	1	3	12	15	
124	2	2	11	12	
125	2	1	5	9	
126	1	1 - 2	6	15	
127	—	3	—	—	No parasitic relapse for 60 days. Continuous low intermittent fever for 15 days
128	8	Present throughout	—	—	Temperature uncontrolled. (*Vide* Chart)
129	3	Present throughout	—	—	No febrile relapse for 18 days
130	2	1 - 2	5	13	(*Vide* Chart)
131	3	Present throughout	—	10	
132	1	3	8 - 9	10	

* The figures given in this column refer to a febrile relapse accompanied by parasites in the blood within 2-3 days. Other non-parasitic febrile attacks are recorded in the 'Remarks' column. This is applicable to all febrile records in this paper.

CASE 128



CASE 130



5-GRAIN (QUININE BIHYDROCHLORIDE) SERIES (Cases 133-150)

Quinine bihydrochloride in solution was given in 5 grain doses on each of two consecutive days in eighteen cases. The results are recorded in Table II.

In seventeen of the eighteen cases parasites disappeared from the blood in one to three days after the first dose; in the remaining one (Case 134, *vide* chart) parasites persisted throughout. In seventeen cases the temperature fell to normal in one to four days after the first dose, while in one case (Case 139) treatment was given during an apyrexial period.

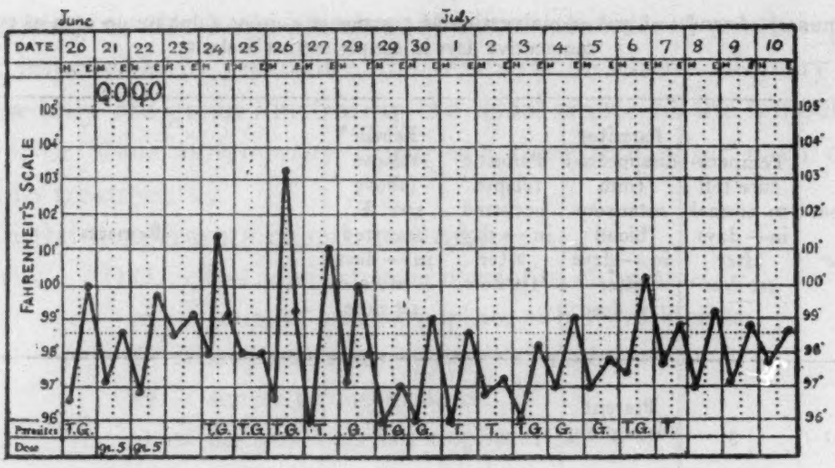
Relapses. Parasitic relapses occurred within three to fifteen days. Febrile relapses occurred in nine to twenty days.

TABLE II.

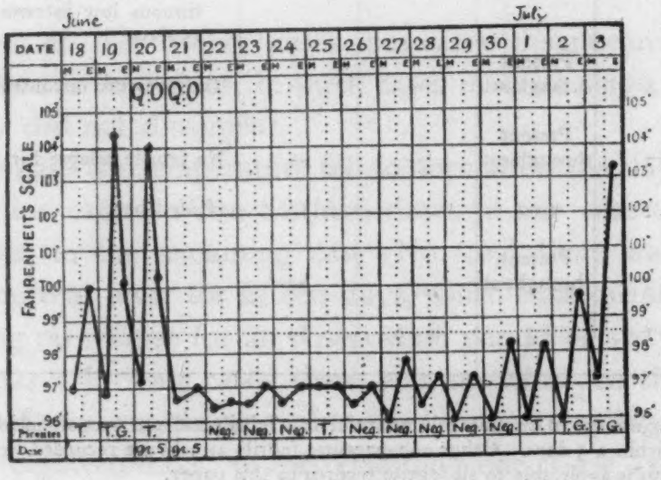
Summary of results of oral administration of 5 grains of quinine bihydrochloride on each of two consecutive days in simple tertian malaria.

Number of case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Parasitic relapse occurred in — days after 1st dose	Febrile relapse (above 100° F.) occurred in — days after 1st dose	Remarks
133	1	2	10	15	
134	2	Present throughout	—	9	(*Vide* Chart)
135	3	3	10	10	
136	2	3	11	11	(*Vide* Chart)
137	4	3	11	11	
138	1	3	14	15	
139	Apyrexia	3	5	20	(*Vide* Chart)
140	2	3	13	16	
141	2	2	14	16	
142	2	2	12	16	
143	2	3	10	11	
144	1	3	8	10	
145	2	2-3	11	11	
146	2	1	13	20	
147	1	1-2	14	19	
148	1	1	3	16	Blood negative 4th to 16th days
149	1	2	14-15	15	
150	2	3	12	—	Quinine orally grains 15 on 13th day

CASE 128



CASE 130



5-GRAIN (QUININE BIHYDROCHLORIDE) SERIES (Cases 133-150)

Quinine bihydrochloride in solution was given in 5 grain doses on each of two consecutive days in eighteen cases. The results are recorded in Table II.

In seventeen of the eighteen cases parasites disappeared from the blood in one to three days after the first dose; in the remaining one (Case 134, *vide* chart) parasites persisted throughout. In seventeen cases the temperature fell to normal in one to four days after the first dose, while in one case (Case 139) treatment was given during an apyrexial period.

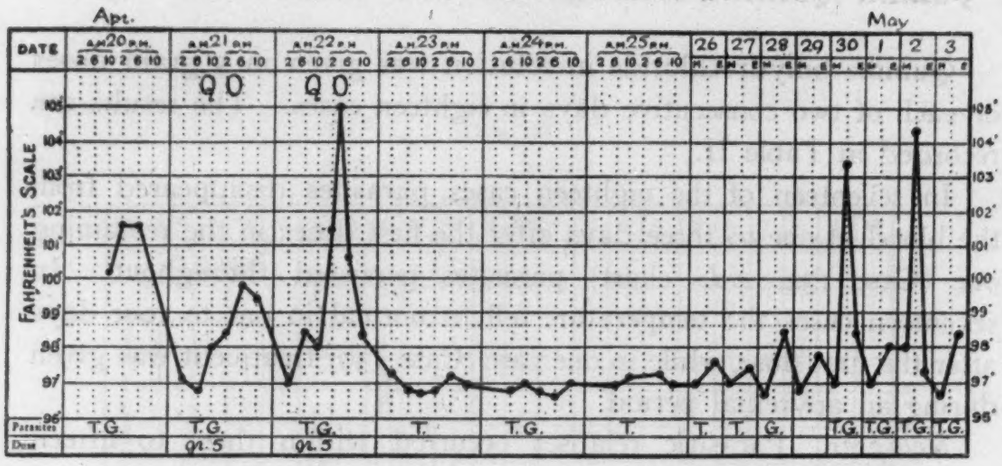
Relapses. Parasitic relapses occurred within three to fifteen days. Febrile relapses occurred in nine to twenty days.

TABLE II.

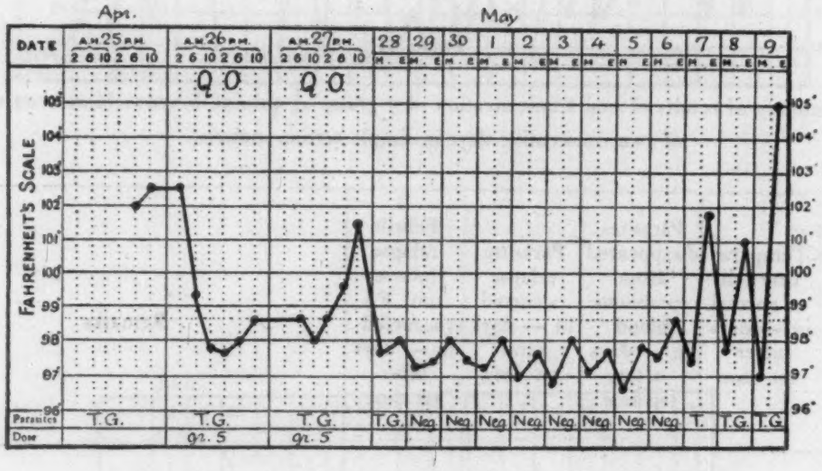
Summary of results of oral administration of 5 grains of quinine bihydrochloride on each of two consecutive days in simple tertian malaria.

Number of case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Parasitic relapse occurred in — days after 1st dose	Febrile relapse (above 100° F.) occurred in — days after 1st dose	Remarks
133	1	2	10	15	
134	2	Present throughout	—	9	(*Vide* Chart)
135	3	3	10	10	
136	2	3	11	11	(*Vide* Chart)
137	4	3	11	11	
138	1	3	14	15	
139	Apyrexia	3	5	20	(*Vide* Chart)
140	2	3	13	16	
141	2	2	14	16	
142	2	2	12	16	
143	2	3	10	11	
144	1	3	8	10	
145	2	2-3	11	11	
146	2	1	13	20	
147	1	1-2	14	19	
148	1	1	3	16	Blood negative 4th to 16th days
149	1	2	14-15	15	
150	2	3	12	—	Quinine orally grains 15 on 13th day

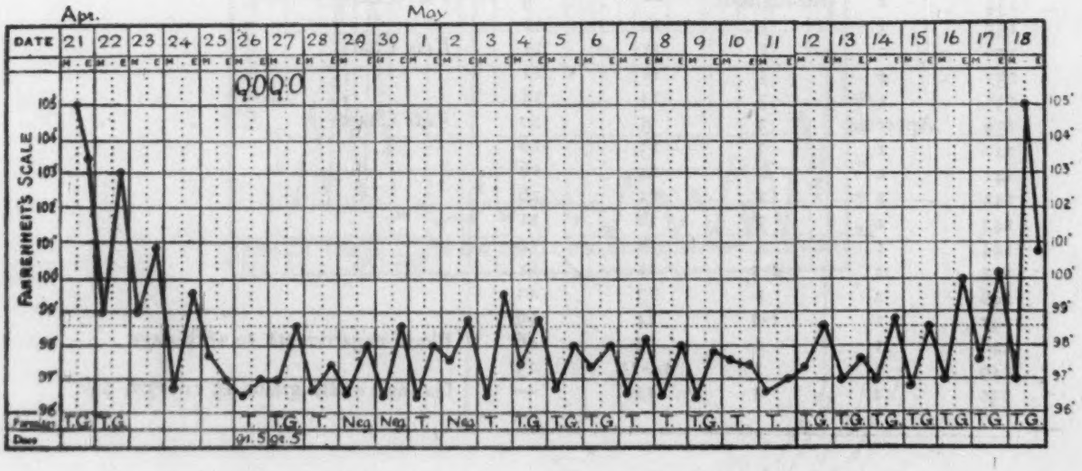
CASE 134



CASE 136



CASE 139



10-GRAIN SERIES (Cases 151-160)

Quinine sulphate in solution was given in 10 grain doses on each of two consecutive days in ten cases. The results are recorded in Table III.

Parasites disappeared from the blood in two to three days after the first dose.

The temperature fell to normal in one to two days after the first dose.

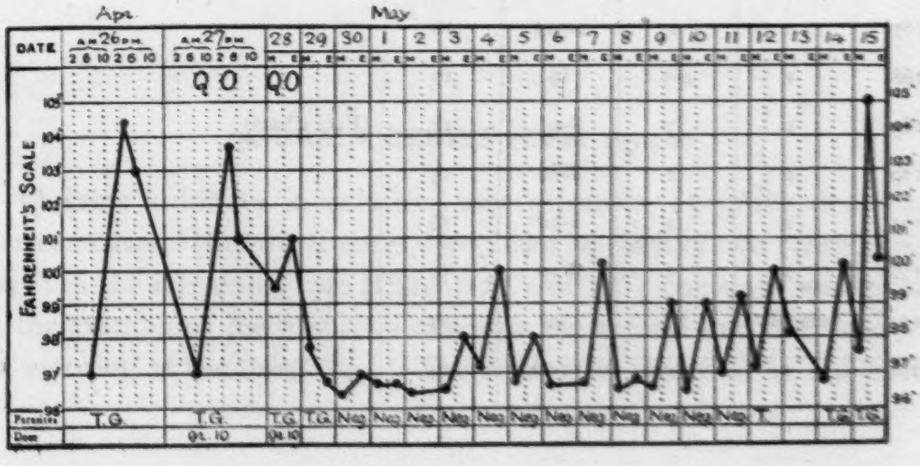
Relapses. Parasitic relapses occurred in ten to eighteen days after the first dose. Febrile relapses occurred in twelve to nineteen days; in one instance (Case 159, *vide* chart) there were two rises of temperature to 100° during the period when the blood was negative.

TABLE III.

Summary of results of oral administration of 10 grains of quinine sulphate on each of two consecutive days in simple tertian malaria.

Number of case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Parasitic relapse occurred in — days after 1st dose	Febrile relapse (above 100° F.) occurred in — days after 1st dose	Remarks
151	1	2	12	16	
152	2	2	13	16	
153	Same day	2	10	12	
154	2	3	18	19	
155	Same day	3	12	12	
156	Same day	2	12	15	
157	2	2	16	19	
158	2	2	12	15	
159	2	3	15	15	100° F. on 7th & 10th days. (*Vide* Chart)
160	1	2	10	12	

CASE 159



15-GRAIN SERIES (Cases 161-174)

Quinine sulphate in solution was given in 15 grain doses on each of two consecutive days in fourteen cases. The results are recorded in Table IV.

Parasites disappeared from the blood in one to five days after the first dose.

The temperature fell to normal in one to two days after the first dose.

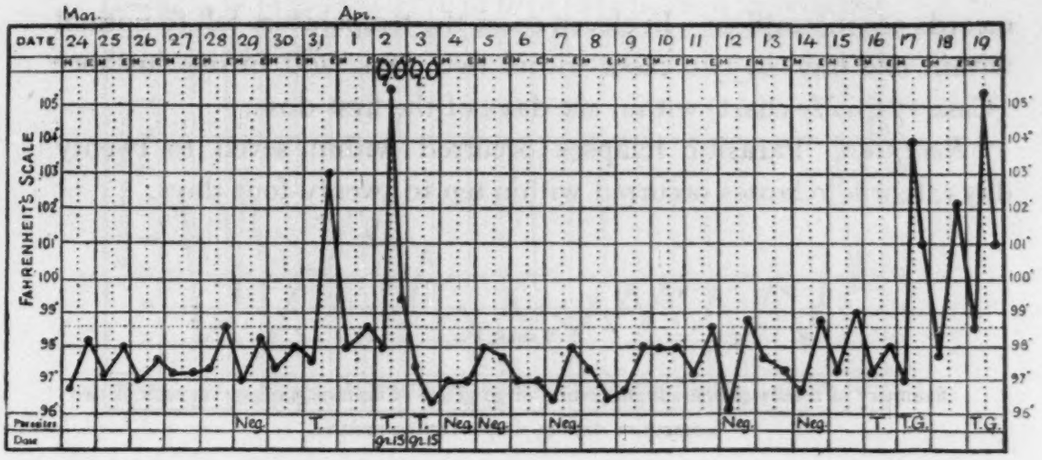
Relapses. Parasitic relapses occurred in eight to twenty-two days; febrile relapses occurred in twelve to twenty-five days.

TABLE IV.

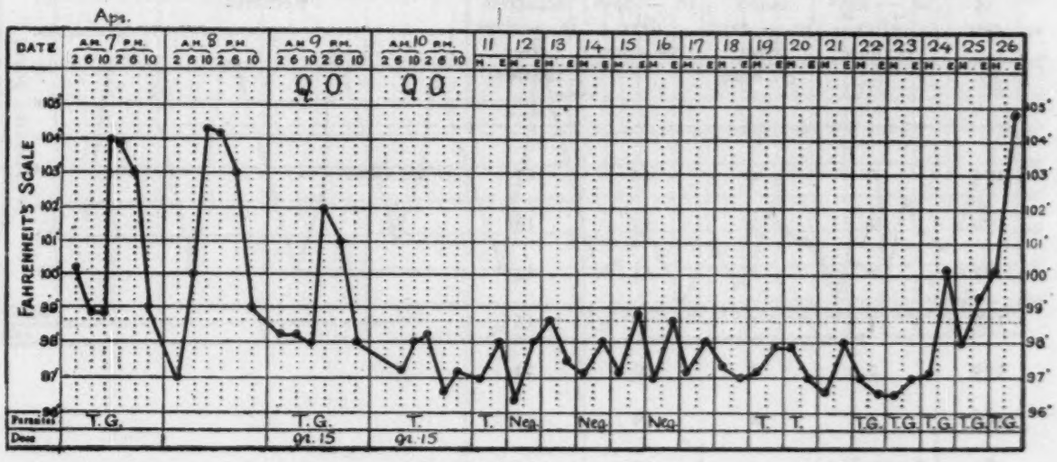
Summary of results of oral administration of 15 grains of quinine sulphate on each of two consecutive days in simple tertian malaria.

Number of case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Parasitic relapse occurred in — days after 1st dose	Febrile relapse (above 100° F.) occurred in — days after 1st dose	Remarks
161	1	2	9-12	12	
162	1	2	9-13	16	
163	2	2	12-14	18	
164	1	2	13-14	15	(*Vide* Chart)
165	Same day	1	22	25	
166	1	4	10-11	14	
167	1	1	12-13	16	
168	1	4-5	16	17	
169	1	3	8-10	15	(*Vide* Chart)
170	1	2	8-10	13	
171	2	2	8-10	16	
172	1	2	8-10	13	
173	Same day	2	11	14	
174	1	4-5	10-11	14	

CASE 164



CASE 169



30-GRAIN SERIES (Cases 175-188)

Quinine sulphate in solution was given in 15 grain doses in fourteen cases. In twelve cases, two doses on each of two consecutive days; in the remaining two (Cases 175 and 176), one dose was given on the evening of the first, two doses on the second and one dose on the morning of the third day. The results are recorded in Table V.

In nine cases parasites disappeared from the blood in one to four days after the first dose; in the remaining five (Cases 184-188) the records are incomplete. In eleven cases the temperature fell to normal within one day, in two cases within two days, and in one instance (Case 175, *vide* chart) within six days of the first dose.

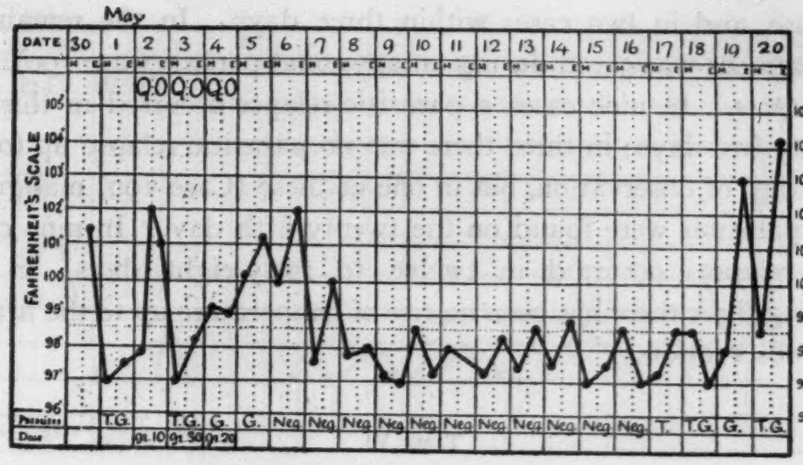
Relapses. Parasitic relapses occurred within seven to twenty days; febrile relapses occurred within ten to twenty-four days.

TABLE V.

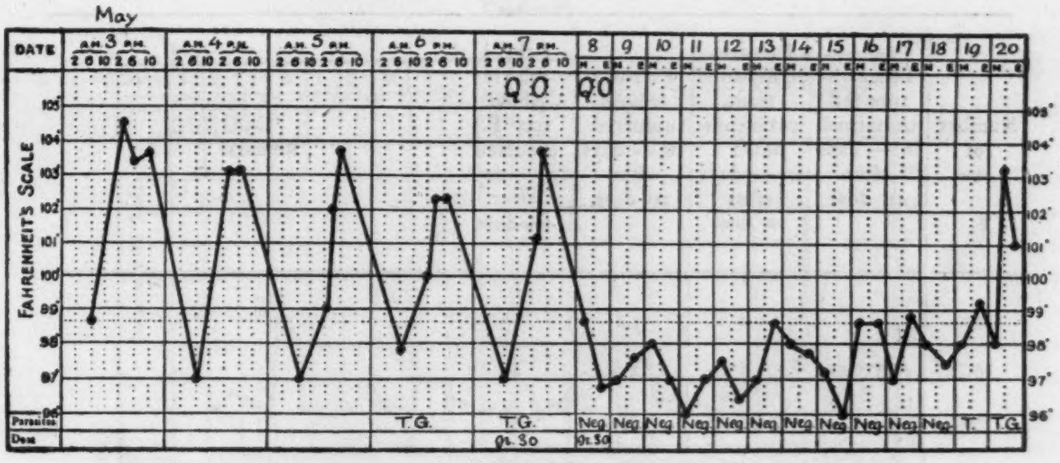
Summary of results of oral administration of 30 grains of quinine sulphate on each of two consecutive days in simple tertian malaria.

Number of case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Parasitic relapse occurred in — days after 1st dose	Febrile relapse (above 100° F.) occurred in — days after 1st dose	Remarks
175	6	4	15	17	(*Vide* Chart)
176	1	3	13	18	
177	2	2	14	24	
178	1	1	7	12	
179	2	2	20	22	
180	1	2	12	15	
181	Same day	2	12	12	
182	1	1	12	13	(*Vide* Chart)
183	1	3	12	14	
184	Same day	1-11	19	19	
185	1	1-7	8-11	10	
186	1	1-7	15	21	
187	1	1-7	12	12	
188	1	2-7	13	15	

CASE 175



CASE 182



45-GRAIN SERIES (Cases 189-200)

Quinine sulphate in solution was given in 15 grain doses in twelve cases; one dose on the evening of the first, three doses on the second and two doses on the third day. The results are recorded in Table VI.

In eleven of the twelve cases parasites disappeared from the blood in one to three days after the first dose. In the twelfth case (a double infection) simple tertian parasites disappeared the day after the first dose, but the crescents persisted (Case 200).

In nine cases the temperature fell to normal within one day of the first dose, and in two cases within three days. In the remaining case treatment was given during an apyrexial period (Case 190).

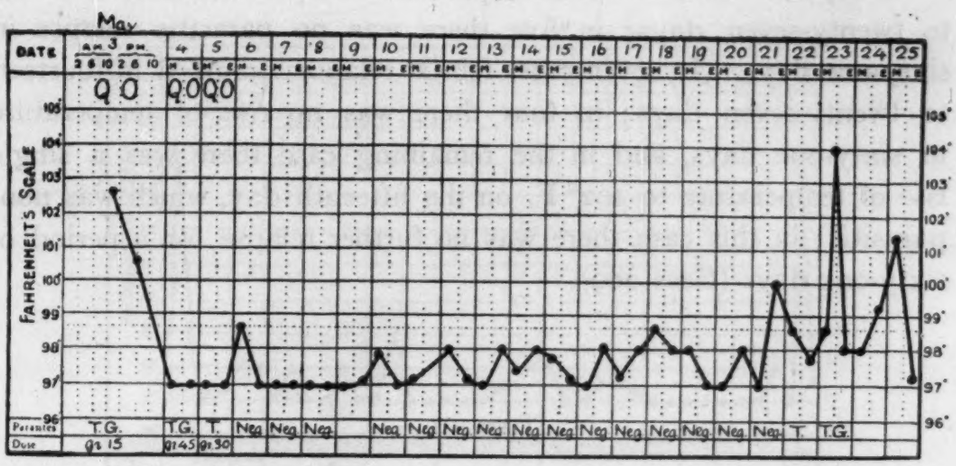
Relapses. In nine cases a parasitic relapse occurred in thirteen to twenty-five days; in three there was no parasitic relapse up to the fiftieth day of observation, but in one of these (Case 199) malignant tertian parasites were found on the twenty-fifth day. In nine cases febrile relapses occurred in twelve to forty-eight days; in the remaining three there has been no rise of temperature up to the fiftieth day of observation.

TABLE VI.

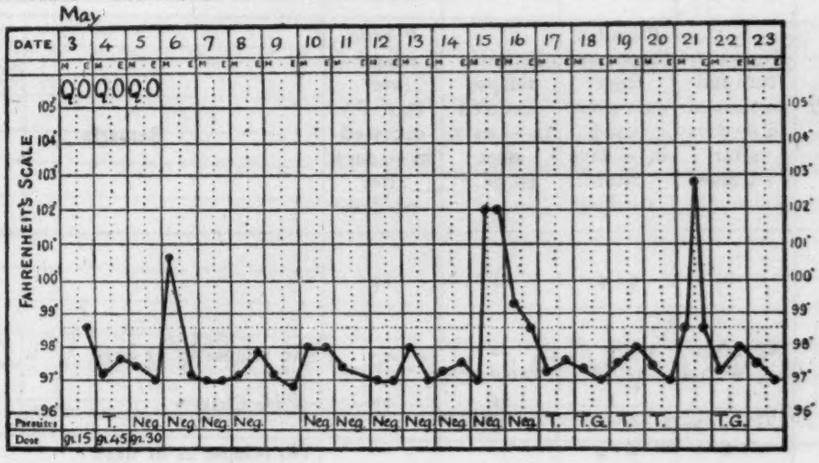
Summary of results of oral administration of 45 grains of quinine sulphate on each of two consecutive days in simple tertian malaria.

Number of case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Parasitic relapse occurred in — days after 1st dose	Febrile relapse (above 100° F.) occurred in — days after 1st dose	Remarks
189	1	1	14	15	
190	Apyrexia	1	21	21	
191	1	3	15	18	
192	1	1	13	15	
193	1	2	—	—	No relapse up to 50th day
194	1	3	19	18	(*Vide* Chart)
195	1	2	25	48	
196	1	1	17	19	
197	1	1	15	15	
198	3	2	14	12	(*Vide* Chart)
199	Same day	2	—	—	Relapsed with malignant tertian trophozoites on 25th day. No relapse of simple tertian malaria in 50 days.
200	3	1	—	—	Malignant tertian gametes present 1st to 15th day after 1st dose. No relapse of simple tertian malaria in 50 days

CASE 194



CASE 198



60-GRAIN SERIES (Cases 201-212)

Quinine sulphate in solution was given in 30 grain doses twice daily on each of two consecutive days in twelve cases. The results are recorded in Table VII.

Parasites disappeared from the blood in one to four days.

In two cases the temperature fell to normal on the day of the first dose, in five cases within one day, in three cases within two; and in the remaining two cases treatment was given during an apyrexial period (Cases 202 and 205).

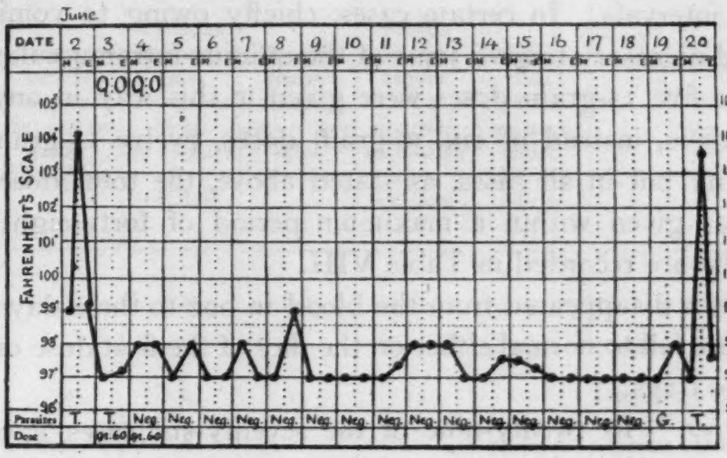
Relapses. In seven cases a parasitic relapse occurred in eleven to twenty-seven days; in five there was no parasitic relapse in sixty-one days. In seven cases febrile relapses occurred in fourteen to twenty-seven days; in four there was no rise of temperature in sixty-one days, and in the remaining case there was a single rise of temperature to 102° F. on the fifteenth day, which was non-parasitic; in this case there was no further relapse for a period of sixty-one days (Case 209).

TABLE VII.

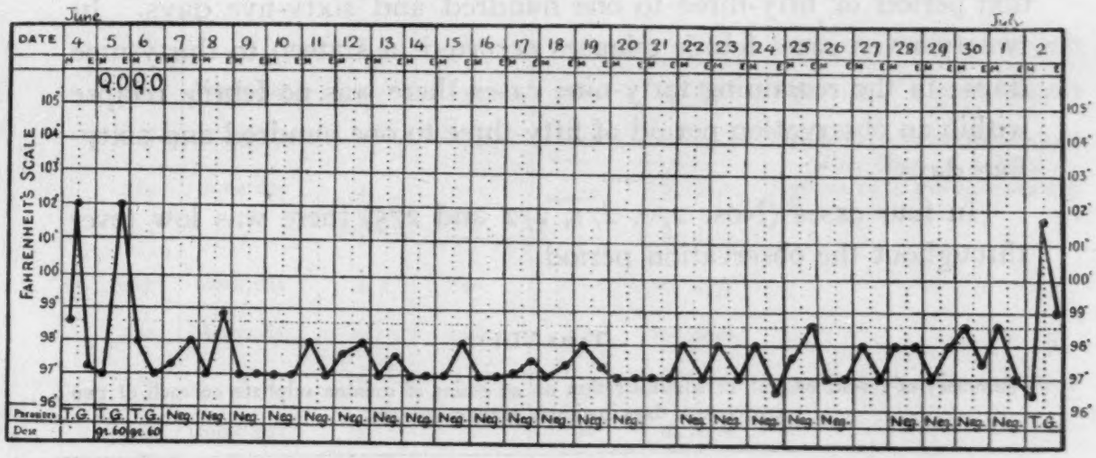
Summary of results of oral administration of 60 grains of quinine sulphate on each of two consecutive days in simple tertian malaria.

Number of Case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Parasitic relapse occurred in — days after 1st dose	Febrile relapse (over 100° F.) occurred in — days after 1st dose	Remarks
201	1	3 - 4	11	14	
202	Apyrexia	1	—	—	No relapse in 61 days
203	1	1	16	17	(*Vide* Chart)
204	2	2 - 4	—	—	No relapse in 61 days
205	Apyrexia	2	—	—	No relapse in 61 days
206	Same day	2	—	—	No relapse in 61 days
207	1	1	16	17	
208	2	2	14	16	
209	2	2	—	—	102° F. on 15th day. No parasitic relapse in 61 days
210	Same day	2	20	21	
211	1	2	27	27	(*Vide* Chart)
212	1	3	18	17	100·5° F. on 12th day

CASE 203



CASE 211



90-GRAIN SERIES (Cases 213-288)

Quinine sulphate in solution was given in doses amounting to 90 grains daily on each of two consecutive days in seventy-six cases.

In all the cases the total dose of 180 grains of quinine was given within a maximum period of forty-eight hours. In most cases the drug was given in three 30-grain doses at 8 a.m., 2 p.m. and 8 p.m. on each of the two days, so that the total quantity of 180 grains was given within thirty-six hours. In the remaining cases, owing to the fact that treatment was not commenced until the afternoon, or because

the patient vomited certain doses, the administration was at irregular intervals. In certain cases, chiefly owing to vomiting, it was found necessary to give some of the quinine intramuscularly (not more than five 15-grain doses were given in this way in any case); in still others, instead of six 30-grain doses, twelve 15-grain doses were given: but in all cases, as stated above, the total dose of 180 grains was given within a maximum period of forty-eight hours. The results are recorded in Table VIII.

Parasites disappeared from the blood in one to three days. The temperature fell to normal either on the day of the first dose or within one to two days.

Relapses. In twenty-nine of the seventy-six cases a parasitic relapse occurred in fourteen to fifty-seven days; in the remaining forty-seven cases there was no parasitic relapse within an observation period of fifty-three to one hundred and sixty-five days. In twenty-seven cases febrile relapses occurred in sixteen to thirty-nine days; in the remaining forty-nine cases there was no febrile relapse within an observation period of fifty-three to one hundred and sixty-five days.

In four cases (Nos. 270, 271, 272 and 275) there was low fever throughout the observation period.

TABLE VIII.[1]

Summary of results of oral ‡‡ administration of 90 grains of quinine sulphate on each of two consecutive days in simple tertian malaria.

Number of case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Parasitic relapse occurred in — days after 1st dose	Febrile relapse (above 100° F.) occurred in — days after 1st dose	Observation period in cases which did not relapse	Remarks
213	Same day	1–3	19	22	—	‡‡grains 15 × 4 intramuscularly
214	1	1–2	—	—	79 days	*
215	2	1–2	21	21	—	
†216	1	1	57	57	—	*
217	1	1–2	20	21	—	‡‡grains 15 × 5 intramuscularly*
218	1	1	—	—	80 days	‡‡grains 15 × 4 intramuscularly; 100·4° F. on 57th day*
219	1	3	—	—	63 days	‡‡grains 15 × 4 intramuscularly

[1] For explanation of reference marks see p. 301.

TABLE VIII—*Continued.*

Number of case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Parasitic relapse occurred in — days after 1st dose	Febrile relapse (above 100° F.) occurred in — days after 1st dose	Observation period in cases which did not relapse	Remarks
220	1	1	16	18	—	
221	1	3	—	—	71 days	‡‡grains 15 × 4 intramuscularly
222	1	2	15	17	—	‡‡grains 15 × 4 intramuscularly
223	Same day	2	—	—	54 days	‡‡grains 15 intramuscularly
224	1	3	—	—	58 days	‡‡grains 15 × 4 intramuscularly*
225	1	1	—	—	53 days	‡‡grains 15 intramuscularly
226	1	2	20	19	—	‡‡grains 15 × 4 intramuscularly
227	Same day	2	—	—	140 days	‡‡grains 15 × 4 intramuscularly
228	1	1	25	26	—	‡‡grains 15 × 3 intramuscularly (*Vide* Chart, p. 305)
229	Same day	3	—	—	82 days	‡‡grains 15 × 4 intramuscularly
230	1	2	—	—	65 days	
231	Same day	2	—	—	101 days	
232	Apyrexia	1	—	—	102 days	*
233	1	3	—	—	74 days	‡‡grains 15 × 4 intramuscularly
234	Same day	1	—	—	64 days	*
235	1	2	—	—	62 days	*
236	Same day	2	23	24	—	**
237	Same day	1	—	—	69 days	
238	Same day	1	23	30	—	
†††239	Same day	2	38	39	—	
240	1	1	—	—	65 days	102° F. on 32nd day; 100·8° F. on 34th day; 100·6° F. on 55th day**
241	1	1	—	—	60 days	100° F. on 42nd day
†242	Same day	1	22	24	—	
243	2	2–3	—	—	65 days	100° F. on 32nd day; 100° F. on 55th day**
244	Same day	2	—	—	65 days	*
245	Apyrexia	1	—	—	75 days	**

TABLE VIII—*Continued.*

Number of case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Parasitic relapse occurred in — days after 1st dose	Febrile relapse (above 100° F.) occurred in — days after 1st dose	Observation period in cases which did not relapse	Remarks
246	Same day	2	16	20	—	‡‡grains 15 intramuscularly*
247	1	2	19	22	—	‡‡grains 15 × 4 intramuscularly
248	—	2	—	—	71 days	
249	Apyrexia	3	—	—	74 days	
250	—	2	—	—	65 days	
251	2	2	19	19	—	‡‡grains 15 intramuscularly
252	—	1	—	—	77 days	
253	—	1	—	—	65 days	
254	1	2	—	—	62 days	‡‡grains 15 intramuscularly
255	—	1–2	—	—	66 days	
256	—	1	—	—	69 days	
257	1	3	—	—	53 days	
258	1	1	22	22	—	
259	—	1	—	—	85 days	**
260	—	2	—	—	66 days	
261	—	1	32	33	—	
262	Same day	2	—	—	65 days	
†263	Apyrexia	1	17	19	—	
264	Same day	1	—	—	80 days	101° F. on 11th day; 100° F. on 26th and 27th days
265	Apyrexia	1	16	18	—	
266	1	1	15	16	—	
267	Apyrexia	1	14	—	—	Parasites found on 14th, 15th, 21st, 22nd, 23rd, 24th and 30th days, but not subsequently. Discharged on 78th day. No temperature during whole of 78 days (*vide* chart)
268	1	1	—	—	78 days	100° F. on 25th day
269	Same day	1	20	23	—	

TABLE VIII—*Continued.*

Number of case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Parasitic relapse occurred in — days after 1st dose	Febrile relapse (above 100° F.) occurred in — days after 1st dose	Observation period in cases which did not relapse	Remarks
270	Same day	1	—	—	165 days	Low, irregular temperature throughout
271	—	1	—	—	164 days	Irregular temperature throughout (*vide* chart)
272	—	1	—	—	93 days	Low, irregular temperature throughout (*vide* chart)
273	Apyrexia	1	—	—	75 days	
††274	Same day	1	20	22	—	
275	—	2	24	25	—	Irregular temperature throughout (*vide* chart)
276	Same day	2	—	—	99 days	100° F. on 72nd day; 101° F. on 73rd day
277	Same day	1	—	—	73 days	*
278	Same day	1	27	30	—	
279	Apyrexia	1	34	34	—	
280	1	1	—	—	64 days	*
281	1	1	—	—	77 days	
282	Same day	1	17	18	—	
283	Same day	1	—	—	80 days	*
284	Same day	1	—	—	67 days	
285	2	1	—	—	73 days	**
286	1	1	—	—	78 days	100° F. on 49th, 50th, and 51st days
287	1	1	18	19	—	
288	Apyrexia	1	17	19	—	

† Contracted the infection in Mesopotamia.

†† Contracted the infection in France.

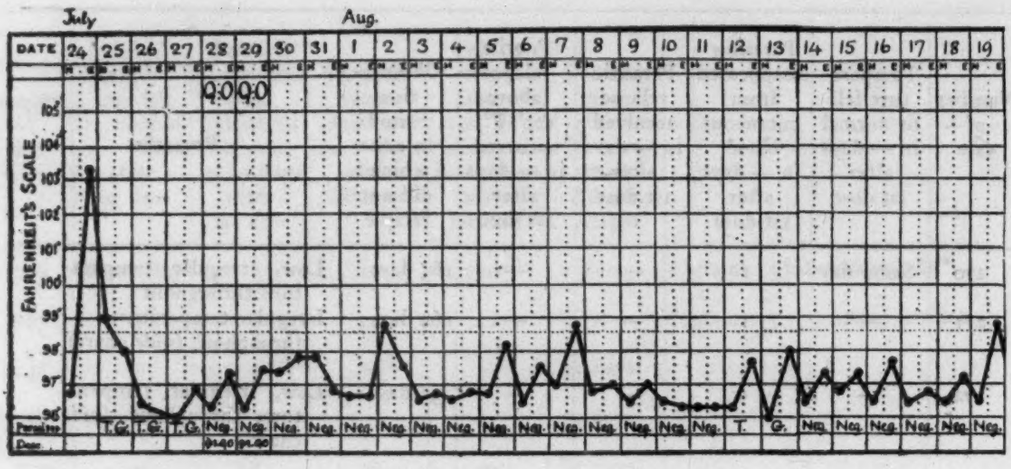
††† Contracted the infection in India.

* Postcards were issued to the 47 cases that had not relapsed on discharge from hospital, with a request to notify whether 30 days later they had or had not relapsed. They were requested to take no quinine after discharge. Replies were received from 19 cases. In 13 cases (*) no relapses and in 6 cases (**) relapses were recorded, but whether these latter were parasitic there was no means of ascertaining.

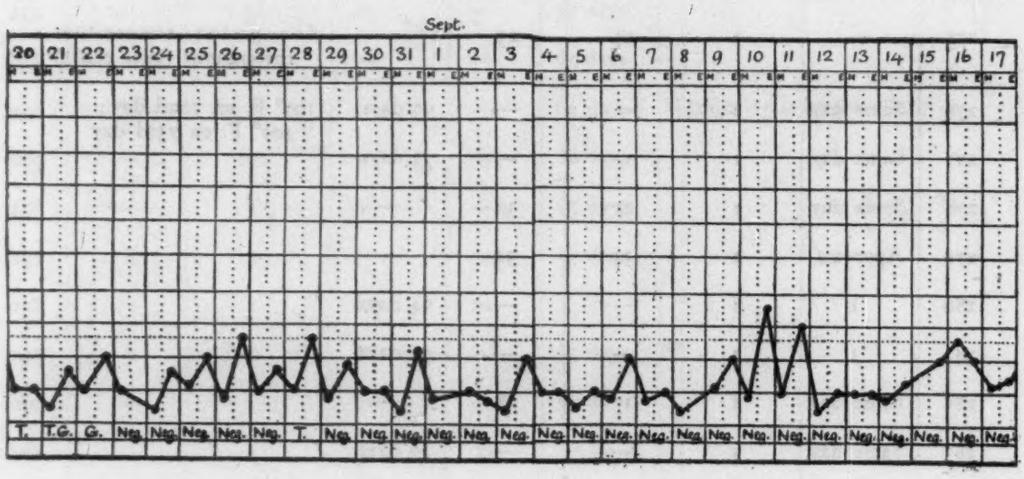
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‡‡ As explained in the text, in certain cases a part of the total dose of 180 grains was given intramuscularly.

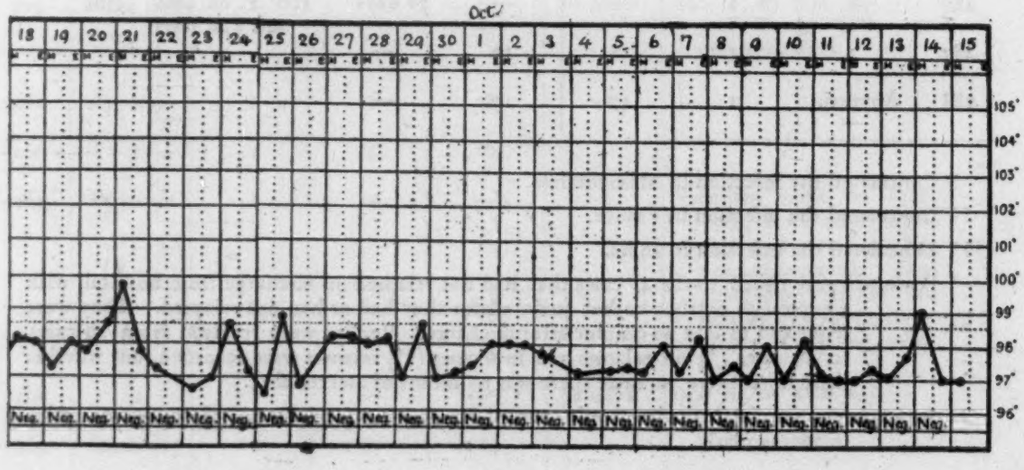
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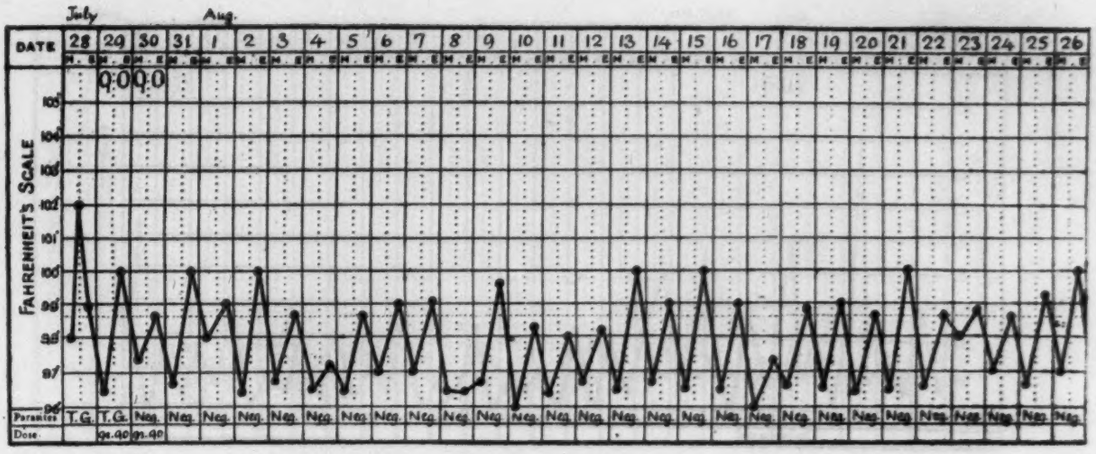
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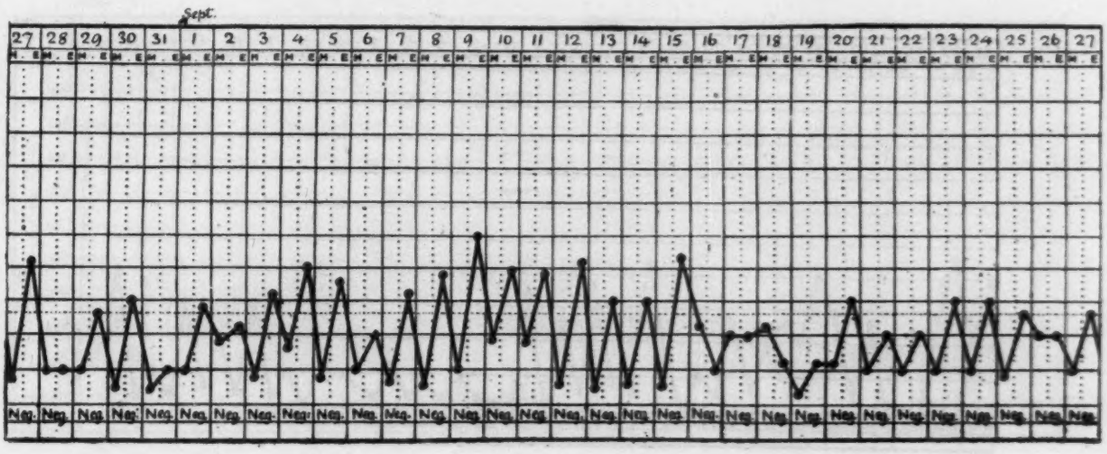
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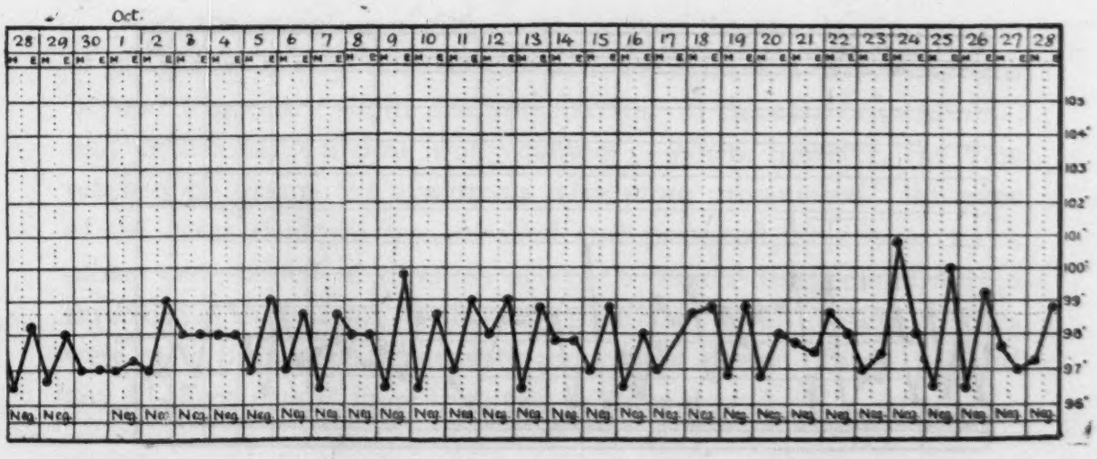
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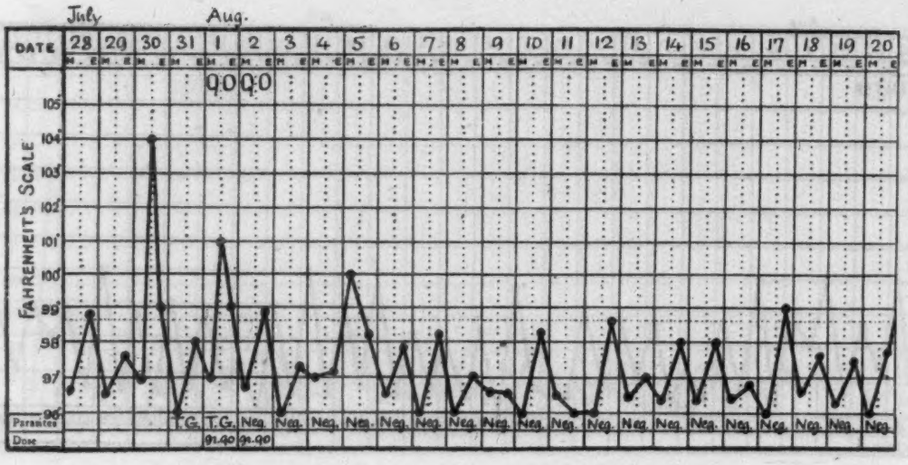
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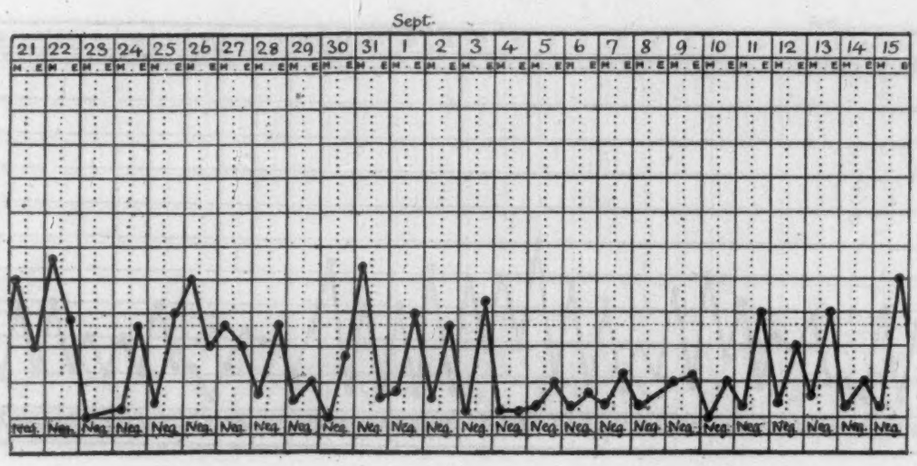
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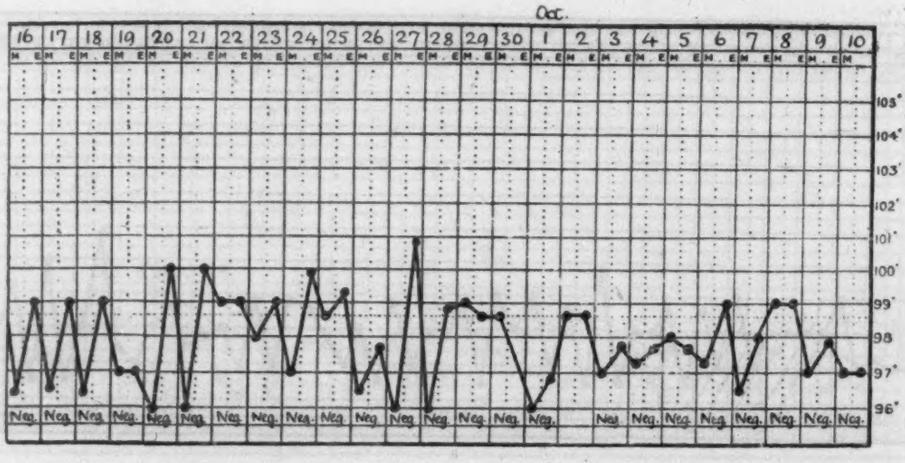
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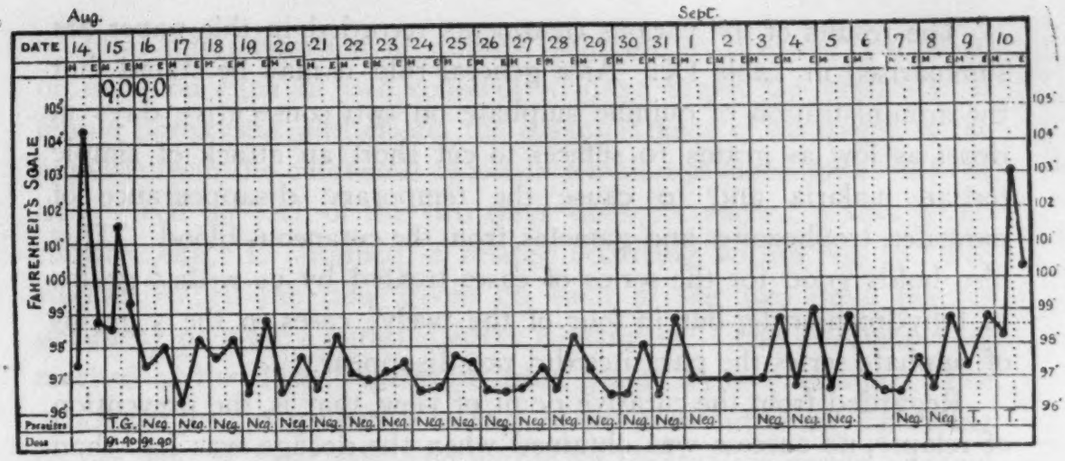
CASE 272 (Part II)



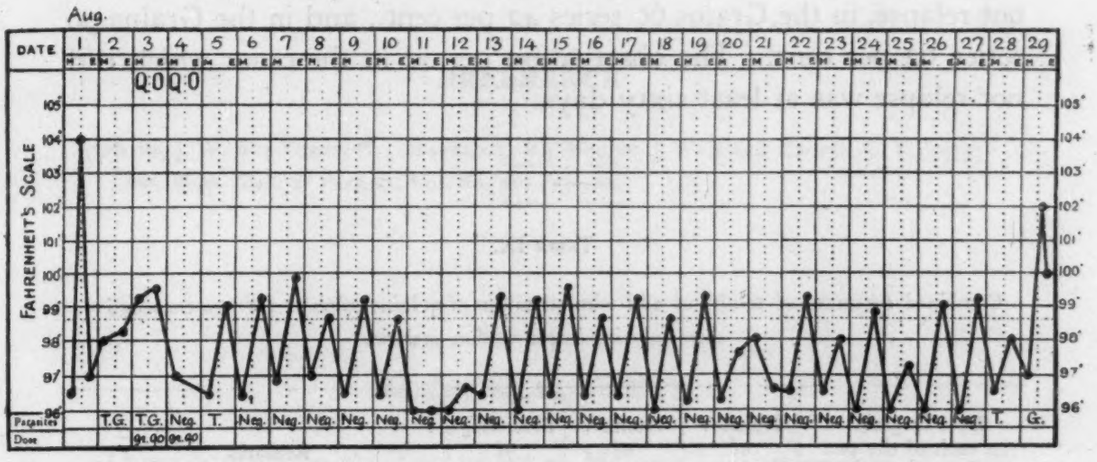
CASE 272 (Part III)



CASE 228



CASE 275



TOLERANCE OF TREATMENT

With the exception of those who received the two days' treatment of 90 grains a day, none of the patients exhibited any symptoms beyond temporary slight deafness, buzzing in the ears or giddiness; very occasionally a dose was vomited. When, however, the dose reached 90 grains the symptoms were more pronounced. In some of the cases deafness was marked and persisted for several days, others complained of dimness of vision, which disappeared within three days; the buzzing in the ears, giddiness, vomiting and tremors were more pronounced than with the smaller doses. In no case did any ill-effect persist more than two or three days.

SUMMARY

The results of the various treatments recorded in this paper are summarised in Table IX. As a general rule, it may be stated that the administration of quinine sulphate on two consecutive days in doses as low as grains 10 suffices to cut short an attack of simple tertian malaria and to cause the temporary disappearance of parasites, trophozoites and gametes, from the cutaneous blood. This also holds good for the series of cases treated by us with 5 grains of bihydrochloride, but in four of the twelve cases in the 5 grains of sulphate series the parasites did not disappear.

Regarded from the curative point of view, that is, the prevention of relapse, no success was obtained when the dosage was less than grains 45. When this dosage was reached a curative value became appreciable: thus in the Grains 45 series 25 per cent. of cases did not relapse, in the Grains 60 series 42 per cent., and in the Grains 90 series 62 per cent. The observation period in the cases which did not relapse was at least sixty days.

TABLE IX.

Combined summary of results of oral administration of quinine sulphate for two consecutive days only in simple tertian malaria.

Dose in grains on each of the two consecutive days	Number of cases	Number of relapses	Percentage of relapses	Remarks
5 sulphate	12	11	91·6	In 4 of these cases there was no effect on fever or parasites
5 bihydrochloride	18	18	100	In 1 of these cases there was no effect on fever or parasites
10 sulphate	10	10	100	
15 sulphate	14	14	100	
30 sulphate	14	14	100	
45 sulphate	12	9	75	In the 3 cases which did not relapse the observation period was only 50 days.
60 sulphate	12	7	58	
90 sulphate	76	29	38·1	

CONCLUSIONS

Oral administration of quinine sulphate in doses of grains 10 or more on each of two consecutive days causes the cessation of febrile paroxysms and effects the disappearance of all stages of the parasite from the cutaneous blood in simple tertian malaria. If the dose given on each of the two days does not exceed grains 30 no curative effect is obtained, a relapse occurring within two to three weeks. When the dose given on each of the two days reaches grains 45 or more a curative effect is manifest. This becomes more marked as the dose is increased from grains 45 to grains 90. The maximum dose of grains 90 on each of two consecutive days prevents 62 per cent. of cases relapsing within an observation period varying from fifty-three to one hundred and sixty-five days.

REFERENCE

STEPHENS, J. W. W.; YORKE, W.; BLACKLOCK, B.; MACFIE, J. W. S. and COOPER, C. F. (1917). *Ann. Trop. Med. & Parasit.*, Vol. XI, pp. 113-125.

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These Studies in the Treatment of Malaria (a continuance of work already in progress) were undertaken at the request of, and part of the expenses in connection therewith are defrayed by, the War Office. We regret that this acknowledgment was inadvertently omitted from the introduction to this series of papers.

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1905	Caldwell, Thomas Cathcart	1906	Sampey, Alexander William
1905	Critien, Attilio	1906	Smithson, Arthur Ernest
1905	Hooton, Alfred	1906	Taylor, Joseph van Someron
1905	Hudson, Charles Tilson	1906	Taylor, William Irwin
1905	Illington, Edmund Moritz	1906	Tynan, Edward Joseph

Date of Diploma	
1906	Watson, Cecil Francis
1906	Willcocks, Roger Durant
1906	Williamson, George Alexander
1907	Allan, Alexander Smith
1907	Allwood, James Aldred
1907	Bond, Ashton
1907	Branch, Stanley
1907	Collinson, Walter Julius
1907	Davey, John Bernard
1907	Donaldson, Anson Scott
1907	Fell, Matthew Henry Gregson
1907	Gann, Thomas William Francis
1907	Graham, James Drummond
1907	Hiscock, Robert Carroll
1907	Keane, Joseph Gerald
1907	Kennan, Richard Henry
1907	Kenrick, William Hamilton
1907	Le Fanu, George Ernest Hugh
1907	Mackey, Charles
1907	Maddox, Ralph Henry
1907	McCarthy, John McDonald
1907	Raikes, Cuthbert Taunton
1907	Ryan, Joseph Charles
1907	Vallance, Hugh
1908	Caverhill, Austin Mack
1908	Crawford, Gilbert Stewart
1908	Dalal, Kaikhusroo Rustomji
1908	Dansey-Browning, George
1908	Davidson, James
1908	Dickson, John Rhodes
1908	Dowdall, Arthur Melville
1908	Glover, Henry Joseph
1908	Greaves, Francis Wood
1908	Goodbody, Cecil Maurice
1908	Harrison, James Herbert Hugh
1908	Joshi, Lemuel Lucas
1908	Le Fanu, Cecil Vivian
1908	Luethgen, Carl Wilhelm Ludwig
1908	Mama, Jamshed Byramji
1908	McCay, Frederick William
1908	McLellan, Samuel Wilson
1908	Pearce, Charles Ross
1908	Schoorel, Alexander Frederik
1908	Smith, John Macgregor
1908	Stewart, George Edward
1908	Tate, Gerald William
1908	Whyte, Robert
1909	Abercrombie, Rudolph George
1909	Allin, John Richard Percy
1909	Armstrong, Edward Randolph
1909	Barrow, Harold Percy Waller
1909	Beatty, Guy
1909	Carr-White, Percy
1909	Chevallier, Claude Lionel
1909	Clark, William Scott
1909	Cope, Ricardo
1909	Fleming, William
1909	Hanschell, Hother McCormick
1909	Hayward, William Davey
1909	Henry, Sydney Alexander
1909	Innes, Francis Alexander

Date of Diploma	
1909	Jackson, Arthur Frame
1909	Kaka, Sorabji Manekji
1909	McCabe-Dallas, Alfred Alexander Donald
1909	Meldrum, William Percy
1909	Murphy, John Cullinan
1909	Samuel, Mysore Gnananandaraju
1909	Shroff, Kawasjee Byramjee
1909	Thornely, Michael Harris
1909	Turkhud, Violet Ackroyd
1909	Webb, William Spinks
1909	Yen, Fu-Chun
1910	Brabazon, Edward
1910	Castellino, Louis
1910	Caulcrick, James Akilade
1910	Dowden, Richard
1910	Haigh, William Edwin
1910	Hamilton, Henry Fleming
1910	Hefferman, William St. Michael
1910	Hipwell, Abraham
1910	Homer, Jonathan
1910	Houston, William Mitchell
1910	James, William Robert Wallace
1910	Johnstone, David Patrick
1910	Korke, Vishnu Tatyaji
1910	Macdonald, Angus Graham
1910	Macfie, John Wm. Scott
1910	Manuk, Mack Walter
1910	Murison, Cecil Charles
1910	Nanavati, Kishavlal Balabhai
1910	Nauss, Ralph Welty
1910	Oakley ,Philip Douglas
1910	Pratt, Ishmael Charles
1910	Sabastian, Thiruchelvam
1910	Shaw, Hugh Thomas
1910	Sieger, Edward Louis
1910	Sousa, Pascal John de
1910	Souza, Antonio Bernardo de
1910	Waterhouse, John Howard
1910	White, Maurice Forbes
1911	Blacklock, Breadalbane
1911	Brown, Frederick Forrest
1911	Chand, Diwan Jai
1911	Holmes, John Morgan
1911	Ievers, Charles Langley
1911	Iles, Charles Cochrane
1911	Ingram, Alexander
1911	Kirkwood, Thomas
1911	Knowles, Benjamin
1911	Liddle, George Marcus Berkeley
1911	Lomas, Emanuel Kenworthy
1911	Mackarell, William Wright
1911	MacKnight, Dundas Simpson
1911	Mascarenhas, Joseph Victor
1911	Murray, Ronald Roderick
1911	Oluwole, Akidiya Ladapo
1911	Rao, Koka Ahobala
1911	Sinton, John Alexander
1911	Tarapurvalla, Byramji Shavakshah
1911	Taylor, John Archibald
1911	Woods, William Medlicott

Date of Diploma	
1912	Aeria, Joseph Reginald
1912	Anderson, Edmund Litchfield
1912	Borle, James
1912	Bowie, John Tait
1912	Brassey, Laurence Percival
1912	Christie, David
1912	Dillon, Henry de Courcy
1912	Dunn, Lillie Eleanor
1912	Hardwicke, Charles
1912	Jagose, Jamshed Rustomji
1912	Kochhar, Mela Ram
1912	McGusty, Victor William Tighe
1912	Milne, Arthur James
1912	Mitra, Manmatha Nath
1912	Myles, Charles Duncan
1912	Pelly, Huntly Nevins
1912	Prasad, Bindeshwari
1912	Prentice, George
1912	Ross, Frank
1912	Russell, Alexander James Hutchison
1912	Ruthven, Morton Wood
1912	Sandilands, John
1912	Seddon, Harold
1912	Smalley, James
1912	Strickland, Percy Charles Hutchison
1912	Watson, William Russel
1913	Austin, Charles Miller
1913	Banker, Shiavux Sorabji
1913	Becker, Johann Gerhardus
1913	Carrasco, Milton
1913	Clark, James McKillican
1913	Forsyth, Charles
1913	Grahame, Malcolm Claude Russell
1913	Grieve, Kelburne King
1913	Hargreaves, Alfred Ridley
1913	Hepper, Evelyn Charles
1913	Hiranand, Pandit
1913	Jackson, Oswald Egbert
1913	Khaw, Ignatius Oo Kek
1913	MacKelvie, Maxwell
1913	MacKinnon, John MacPhail
1913	Macmillan, Robert James Alan
1913	Mouat-Biggs, Charles Edward Forbes
1913	Noronha, John Carmel
1913	O'Connor, Edward
1913	Olubomi-Beckley, Emanuel
1913	Pestonji, Ardeshir Behramshah
1913	Puttanna, Dodballapur Sivappa
1913	Reford, John Hope
1913	Smith, Edward Arthur
1913	Stewart, Samuel Dudley
1913	Walker, Frederick Dearden
1913	Wilbe, Ernest Edward
1913	Wilson, Hubert Francis
1913	Yin, Ulg Ba
1913	Young, William Alexander
1914	Arculli, Hassan el
1914	Chohan, Noormahomed Kasembha
1914	Connell, Harry Bertram
1914	Gerrard, Herbert Shaw
1914	Gimi, Hirji Dorabji
1914	Gwynne, Joseph Robert
1914	Hodkinson, Samuel Paterson
1914	Jackson, Arthur Ivan
1914	Kaushash, Ram Chander
1914	Kelsall, Charles
1914	Luanco y Cuenca, Maximino
1914	Misbah, Abdul-Ghani Naguib
1914	Naidu, Bangalore Pasupulati Balakrishna
1914	Rowe, John Joseph Stephen
1914	Roy, Raghu Nath
1914	Shiveshwarkar, Ramchandra Vishnu
1914	Sur, Sachindra Nath
1914	Talati, Dadabhai Cursedji
1914	Wilkinson, Arthur Geden
1914	Wright, Ernest Jenner
1915	Lobo, John Francis
1915	Madhok, Gopal Dass
1915	Pearson, George Howorth
1915	Swami, Karumuri Virabhadra
1915	Wood, John
1916	Barseghian, Mesroob
1916	Chaliha, Lakshmi Prasad
1916	Lim, Albert Liat Juay
1916	Lim, Harold Liat Hin
1916	Metzger, George Nathaniel
1916	Söderström, Erik Daniel
1916	Wheeler, Louis
1917	Chapman, Herbert Owen
1917	Krishnamoorthy, Yedatore Venkoba
1917	Lipkin, Isaac Jacob
1918	Watts, R. C.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY

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Plates and illustrations should be accompanied by short explanations.

References to authors in the text must be made in the following way:—'According to Smith (1900) the spleen is enlarged, but Robinson (1914) says the reverse.' The references should be collected in alphabetical order of authors' surnames at the end of the paper, and arranged in the following way:—

ROBINSON, S. (1914). 'The spleen in malaria.' *Annals of Nosology*, Vol. XX, pp. 20-25.

SMITH, J. (1900). 'Enlargement of the spleen in malaria.' *Journal of Pathometry*, Vol. I, pp. 1-20.

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ADDENDA AND CORRIGENDA

Page 109, line 23. For 'SINGLE' *read* 'SIMPLE.'

Page 156, line 4 from foot. For 'Febrile paroxysms recurred on an average in 11 days (minimum 11, maximum 15)' *read* '12 days (minimum 9, maximum 18).'

Page 175, footnote. For 'This case relapsed' *read* 'Patient states that he relapsed.'

Page 298, case 216. Delete asterisk at end of line.

Page 299, case 236. Delete double asterisk at end of line.

Page 300, case 246. Delete asterisk at end of line.

" " case 248. In first column, now blank, *read* 1.

" " case 250. " " " " " " 1.

" " case 252. " " " " " " same day.

" " case 253. " " " " " " same day.

" " case 255. " " " " " " same day.

" " case 256. " " " " " " same day.

" " case 259. " " " " " " same day.

" " case 260. " " " " " " 1.

" " case 261. " " " " " " same day.

Page 301, line 5 from foot. For 'Replies were received from 19 cases,' etc. *read* 'Replies were received from 16 cases. In 11 cases (*) no relapses and in 5 cases (**) relapses were recorded,' etc.

Page 306, Table IX, line beginning '45 sulphate,' for '12 | 9 | 75' *read* '12 | 9 or possibly 12 | 75 or possibly 100' [*vide* p. 329].

This slip to be tipped on p. 1, Vol. XI.

STUDIES IN THE TREATMENT OF MALARIA

VII. ORAL ADMINISTRATION OF QUININE SULPHATE DAILY OVER PROLONGED PERIODS, IN SIMPLE TERTIAN MALARIA

BY

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Undertaken at the request of the War Office

(*Received for publication* 25 *September*, 1917)

The primary object of the work recorded in this paper was to determine the curative value of various modes of prolonged quinine treatment similar to those which have from time to time been recommended by various authors. Although these are very numerous—'there are as many plans for the treatment of malaria as there are authorities' (Barlow, 1915)—it is frequently impossible to ascertain from the literature any precise information on the following points of vital importance:—(1) number of cases treated; (2) species of parasite present; (3) records of microscopical examination; (4) length of observation period after treatment.

We shall discuss briefly those treatments in support of which experimental evidence is supplied by their advocates, and ignore those which, so far as one can ascertain, are based on no definite

evidence, but are merely the results of impressions, and which consequently it is impossible to appraise critically.

1. Thomson (1917).

No. of cases treated:—200 (probably infected mainly in West Africa, but 20 in the Amazons)

Species of parasite present:—Malignant tertian in 'the majority.'

Nature and duration of treatment:—Quinine in solution orally, grains 30 daily for three weeks.

Observation period:—'The majority of our cases were lost trace of after their departure from hospital.'

Relapses:—'Only one known to have relapsed.'

Conclusion:—'Taking into account, therefore, the fact that we lost touch with many of the patients after they left hospital, I am inclined to believe until it can be proved otherwise that this three weeks' continuous treatment will permanently cure 80 per cent. of all early cases.'

Comment. This belief may be true or false, it is impossible to say, but it is clear that on the facts as presented the conclusion is not warranted for the following reasons:—(1) There is an absence of observation period in the majority of cases. (2) On p. 12 the author writes 'Sir Ronald Ross often recommended that our Liverpool patients should continue to take about fifteen grains of quinine daily for two months after their discharge from hospital.' If any of the 200 cases (and no others are mentioned) followed this advice, then in such cases the duration of treatment was not three weeks but two months and three weeks.

2. Stott (1916).

No. of cases treated:—347 (Soldiers, natives of India).

Species of parasite present:—Diagnosis apparently clinical. No parasitic observations recorded before or after treatment.

Nature and duration of treatment:—Quinine grains 10, thrice daily until the fever had subsided, and subsequently grains 10, four times a week for four months.

Observation period:—8 months.

Relapses:—121 cases re-admitted = 35 per cent. (about). The majority (75 per cent.) of the total re-admissions—some cases were re-admitted several times—occurred *during* treatment.

Comment. The above figures seem to show that the treatment cured 65 per cent. (about) of cases. The chief objection to this conclusion is the absence of systematic parasitic observations before

and after treatment, leaving one in doubt whether all the cases were malaria, and if so what species of parasite was present, and further whether the treatment had really freed them from parasites.

3. Barlow (1915).

No. of cases treated:—580. (Inhabitants of Cuyamel, Honduras).

Species of parasite present:—Malignant tertian (diagnosed microscopically).

Nature and duration of treatment:—'Preliminary laxative, usually calomel, to be followed by a small dose of magnesium sulphate.
Twenty to 30 grains of quinine bisulphate for two days.
Fifteen grains daily, for one month.
Fifteen grains twice a week for two additional months, in cases of æstivo-autumnal infection.
Rest and light diet during the continuance of the symptoms.'

Observation period:—'from 3-6 months have elapsed since the completion of the treatment.'

Relapses—The details are given in the following table:

MALAGNANT TERTIAN CASES

Duration of Treatment	*Patients*	*Relapses*	*Percentage*
Less than 1 month	116	116	100%
1 month	246	91	37%
3 months	218	0	0%

Comment. Grains 15 daily for one month and grains 15 twice a week for two months cure 100 per cent. of malignant tertian cases. Presumably this means that absence of a clinical relapse or admission to hospital is taken to be equivalent to a cure, as there is no mention of blood examinations beyond the original diagnosis.

In addition Barlow treated 85 cases of tertian (simple) malaria of which 24 were pure. 'The pure cases were advised to take one month's treatment of 15 grains daily. There have been three relapses of tertian malaria. None of these took treatment longer than three weeks. Four cases who only followed the treatment for ten days have remained free from relapses. No case which took the full month of treatment has relapsed, but the number of cases is too few to draw conclusions.'

Comment. No case which took grains 15 daily for one month relapsed.

In the observations recorded in the present paper all the cases were adult males infected in Macedonia at least six months previously, and all had had more or less quinine during this period.

In every instance a diagnosis of simple tertian malaria was made

by microscopical examination: in the vast majority parasites were present in the blood on the day treatment commenced. In the remainder, although a blood examination was not made on the first day of treatment, parasites were present 2-3 days previously. Quinine sulphate in solution was given daily in two or more 10 or 15 grain doses. The cases are grouped according to the total daily dose given.

We shall consider the results: (1) from the palliative point of view, i.e. the degree to which the symptoms are controlled and the blood kept free from parasites during the treatment, and (2) from the curative point of view, i.e., whether or no relapses occurred during the observation period after the cessation of treatment. So far as possible we endeavoured to observe the patients for a period of two months after treatment was discontinued. The results of treatment from both these points of view are shown in the following tables.

Blood examinations, except in the series recorded in Table V, were made once weekly and also whenever the temperature reached 100° F. or over, which in this, as in all our previous papers, is regarded as a febrile paroxysm.

In the following charts and tables.

Numerals 0, 1, 2, etc. = number of parasitic febrile relapses weekly.

Numerals 1*, 2*, etc. = number of non-parasitic febrile attacks weekly.

Numerals 1°, 2°, etc. = number of febrile paroxysms not examined microscopically, weekly.

P = non-febrile parasitic relapse.

Q.O. = quinine sulphate orally.

T. = simple tertian trophozoites or schizonts.

G. = simple tertian gametes.

Neg. = no parasites found.

NOTE.—A rise of temperature above 100° F., of which the nature is unknown, is termed a *febrile attack*. A similar rise of temperature accompanied by parasites in the blood at the time, or within three days, is termed a *parasitic febrile relapse* or *true relapse*. The term *paroxysm* is used indifferently to denote any febrile disturbance of 100° F. or more.

Some of the cases who commenced treatment were unable to tolerate it for eight weeks. For this and other reasons the numbers in Grains 30 and in Grains 45 series diminished from week to week:

for example, in Tables IX and X the experiment commenced with nineteen cases, but at the end of the eighth week only seven remained under treatment. In order, therefore, that the results obtained in the various series of experiments may have a comparative value it is necessary to express the cases having true relapses or febrile attacks as percentages of the total cases undergoing treatment in any particular week. This has been done in Tables IV, VIII and XI, in which the results given in Tables III, VII and X are analysed. By this means the following sets of figures, each having a comparative value, are obtained:—

1. The number of cases which had parasitic febrile relapses each week, expressed as percentages of all cases treated.

2. The number of parasitic febrile relapses experienced per week by each parasitic febrile relapse case.

3. The number of cases which had febrile paroxysms (parasitic and non-parasitic) each week, expressed as percentages of all cases treated.

4. The number of febrile paroxysms (parasitic and non-parasitic) experienced per week by each febrile (parasitic and non-parasitic) case.

The duration of treatment of each case is clearly shown in the tables.

GRAINS 20 SERIES (Cases 289-293)

In these five cases the temperature fell to normal on the day of, or one day after, the commencement of treatment. In four cases parasites disappeared from the cutaneous blood in 1-3 days. In one case parasites did not disappear at the commencement of treatment and were present at intervals throughout the treatment (Case 291).

Relapses.

During treatment. The number of cases (five) observed is too small on which to base a weekly average of relapses.

After treatment. Three of five cases relapsed. Parasites reappeared in 3-10 days after cessation of treatment. Febrile relapses occurred in 9-11 days. It should be noted that in one of the cases that did not relapse the observation period was less than 60 days, viz., 33 days (Table I).

TABLE I.

Results of oral administration of quinine sulphate in solution, grains 20, daily for 14–15 weeks.

Number of Case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Number of febrile paroxysms (parasitic and non-parasitic) and results of blood examinations during treatment — Week of Treatment														Parasitic relapse occurred in — days after cessation of treatment	Febrile relapse (above 100° F.) occurred in — days after cessation of treatment	Observation period in cases which did not relapse
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th			
289	Same day	1–3	0	0	0	0	0	0	0	0	0	0	**1***	**1***	0	0	...	—	—	33 days
290	1	1–3	0	0	0	0	**1***	0	0	0	0	0	0	0	0	0	...	5–9	9	—
291	1	...	**2P**	**P**	**P**	0	0	0	**P**	**P**	**2**	0	0	0	**2**	0	...	3	—	—
292	Same day	1–3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	...	—	—	76 days
293	1	1–3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5–10	11	—

GRAINS 30 SERIES (Cases 294-307)

In these fourteen cases the temperature fell to normal in 1-2 days. In three cases parasites disappeared from the cutaneous blood in 2-5 days, in the remainder examinations were too infrequent to give a precise figure.

Relapses.

During treatment. The average number of cases which had parasitic febrile relapses each week, over a period of eight weeks, was 7·2 per cent. of cases treated, and that of all febrile cases (parasitic and non-parasitic) was 17·3 per cent. (Table IV). The number of cases on which this figure is based is however small, 14 in first week, 8 in eighth week. The chart (Case 306) is a record of a case having five true relapses whilst taking quinine grains 30 daily.

After treatment. Ten of fourteen cases relapsed. Parasites reappeared in 3-49 days and febrile relapses occurred in 5-49 days (Table II).

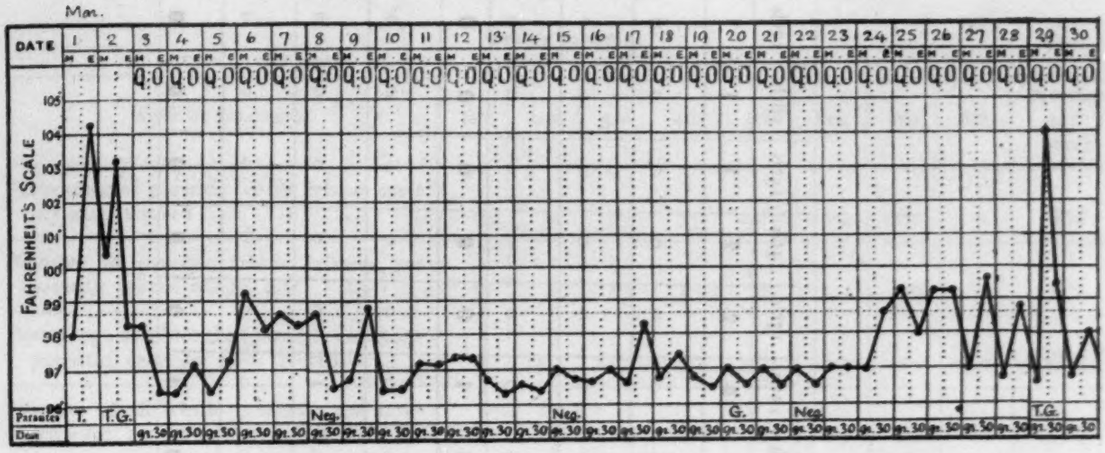
TABLE II.

Results of oral administration of quinine sulphate in solution, grains 30, daily for 5–18 weeks.

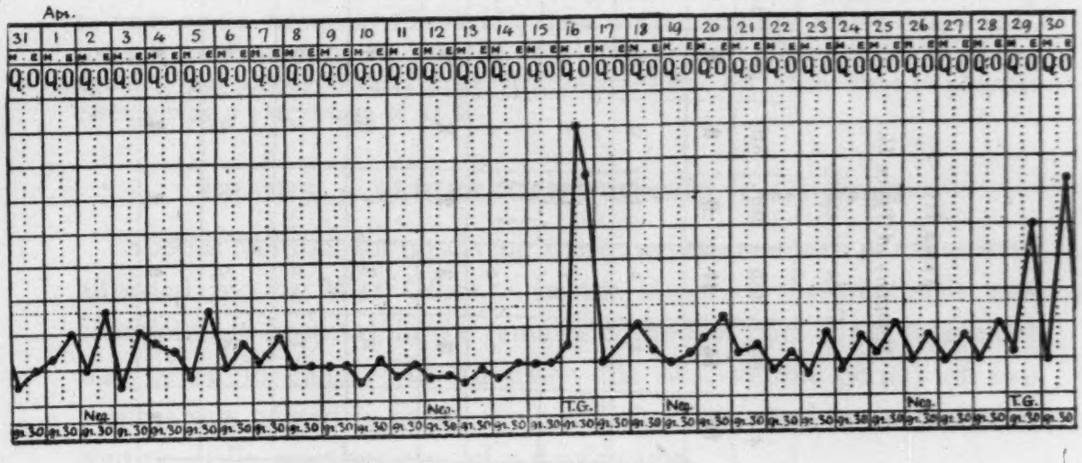
Number of Case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Number of febrile paroxysms (parasitic and non-parasitic) and results of blood examinations during treatment — Week of Treatment																Parasitic relapse occurred in — days after cessation of treatment	Febrile relapse (above 100° F.) occurred in — days after cessation of treatment	Observation period in cases which did not relapse
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	17th	18th			
294	2	2	o	o	o	o	o	...	...	...	...	...	...	...	...	...	...	...	...	...	—	—	61 days
295	2	1–9	o	**1***	**1***	o	o	...	...	...	...	...	...	...	...	...	...	...	...	...	20–21	21	—
296	1	1–5	o	o	o	o	o	...	...	...	...	...	...	...	...	...	...	...	...	...	46–49	49	—
297	1	1–2	o	o	o	o	o	o	...	...	...	...	...	...	...	...	...	...	...	...	5–8	8	—
298	Apyrexia	1–8	o	o	o	o	o	o	...	...	...	...	...	...	...	...	...	...	...	...	31	30	—
299	Apyrexia	1–6	o	o	**1°**	o	o	**1**	o	...	...	...	...	...	...	...	...	...	...	...	7–9	8	—
300	2	2–9	o	o	o	o	o	o	o	o	o	...	...	...	...	...	...	...	...	...	12–16	16	—
301	1	1–10	o	o	o	o	**1***	**2***	**1***	**1***	o	...	...	...	...	...	...	...	...	...	—	—	61 days
302	1	5	o	o	o	o	o	o	o	o	o	...	...	...	...	...	...	...	...	...	—	—	70 days
303	Apyrexia	...	o	**2***	o	o	o	o	o	o	o	...	...	...	...	...	...	...	...	...	7–9	9	—
304	2	1–3	o	o	o	o	**1**	**2**	o	o	**1**	**2**	o	**1***	...	...	...	...	...	...	3	5	—
305	1	2–6	o	o	o	o	o	o	o	**1***	o	o	o	o	...	...	...	...	...	...	18–49†	18–49	—
306	2	2	o	o	**P**	**P**	**1**	o	**1**	o	**3**	**P**	o	o	o	o	...	...	...	...	1–13	13	—
307	1	1–6	o	o	**1**	**1**	o	**P**	o	o	o	o	o	o	o	o	o	o	o	o	—	—	61 days

† Discharged on 18th day after cessation of treatment; re-admitted with malaria on 49th day.

CASE 306 (PART I)



CASE 306 (PART II)



CASE 306 (PART III)

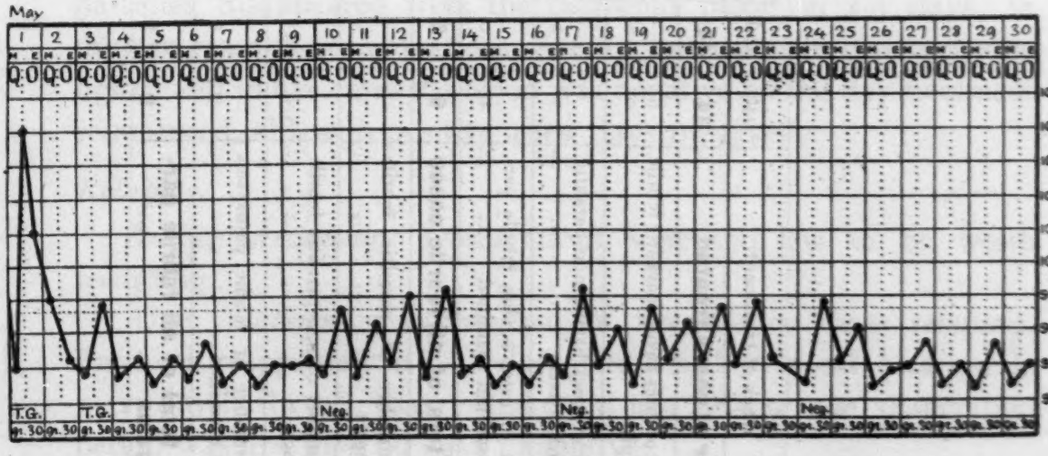


TABLE III.

Summary of TABLE II.

Grains 30 series.

Week of treatment	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	17th	18th
Number of cases treated ...	**14**	**14**	**14**	**14**	**14**	**11**	**9**	**8**	**8**	**4**	**4**	**4**	**2**	**2**	**1**	**1**	**1**	**1**
Number of cases having parasitic febrile relapses	0	0	1	1	2	2	1	0	2	1	0	0	0	0	0	0	0	0
Number of cases having non-parasitic febrile attacks	0	2	1	0	1	1	1	2	0	0	0	1	0	0	0	0	0	0
Number of cases having febrile paroxysms not examined	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Grand total of all febrile cases	**0**	**2**	**3**	**1**	**3**	**3**	**2**	**2**	**2**	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
Total number of parasitic febrile relapses	0	0	1	1	2	3	1	0	4	2	0	0	0	0	0	0	0	0
Total number of non-parasitic febrile attacks	0	3	1	0	1	2	1	2	0	0	0	1	0	0	0	0	0	0
Total number of febrile paroxysms not examined	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Grand total of all febrile paroxysms	**0**	**3**	**3**	**1**	**3**	**5**	**2**	**2**	**4**	**2**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**

TABLE IV.

Analysis of TABLE III, giving the results for the first 8 weeks.

Grains 30 series.

Week of treatment	1st	2nd	3rd	4th	5th	6th	7th	8th	Average per week
Percentage of parasitic febrile relapse cases per cases treated	0	0	7·1	7·1	14·3	18·2	11·1	0	**7·2**
Number of parasitic febrile relapses per parasitic febrile relapse case	0	0	1·0	1·0	1·0	1·5	1·0	0	**0·7**
Percentage of all febrile (parasitic and non-parasitic) cases per cases treated ...	0	14·3	21·4	7·0	21·4	27·3	22·2	25·0	**17·3**
Number of all febrile (parasitic and non-parasitic) paroxysms per febrile case ...	0	1·5	1·0	1·0	1·0	1·7	1·0	1·0	**1·0**

As examples of how the figures in Table IV are obtained we may take 7·1 in the third column of the first line; this signifies that in the third week among 14 cases under treatment (Table III) there was one parasitic febrile relapse case, giving a percentage of 7·1: again, 22·2 in the seventh column of the third line signifies that among 9 cases under treatment (Table III) there were two febrile cases, giving a percentage of 22·2.

GRAINS 30 SERIES—CAMP GROUP (Cases 308-336)

Special facilities for observing these twenty-nine men in camp were afforded by the War Office. In the majority the treatment was completed in hospital, in the remainder the last part of the treatment was given in camp. In all cases the observation period of two months was passed in camp, the men being subjected to the ordinary routine drills, marches, etc., of a depôt camp.

The temperature fell to normal in 1-5 days. In eleven cases parasites disappeared from the cutaneous blood in 1-3 days; in thirteen cases the examinations were too infrequent to give a precise figure, and in five cases the blood was negative on the day treatment commenced, although positive a few days previously.

Relapses.

During treatment. Observations were too few to be worth recording.

After treatment. Twenty-four of twenty-nine cases relapsed. Parasites reappeared in 6-38 days and febrile relapses occurred in 7-41 days. In all cases which did not relapse the observation period was less than 60 days (Table V).

TABLE V.

Results of oral administration of quinine sulphate in solution, grains 30, daily for 8 weeks.

Number of Case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Number of febrile paroxysms (parasitic and non-parasitic) and results of blood examinations during treatment — Week of Treatment								Parasitic relapse occurred in — days after cessation of treatment	Febrile relapse (above 100° F.) occurred in — days after cessation of treatment	Observation period in cases which did not relapse
			1st	2nd	3rd	4th	5th	6th	7th	8th			
308	1	1–7				No	Records				—	—	25 days
309	—	1–3 blood negative									2–8	15	—
310	Apyrexia										26–32	35	—
311	2	2									11–14	14	—
312	1	1–2									6–12	16	—
313	5	2–5									32–38	41	—
314	3	1–3									5–8	8	—
315	1	—									7–13	17	—
316	—	1 blood negative									5–11	15	—
317	Apyrexia										1–6	7	—
318	—	—									—	—	46 days
319	2	—									11–14	14	—
320	—	—									25–31	—	—
321	2–3	1									32	32	—
322	1	1–14									—	—	45 days
323	Apyrexia	2–3									7–12	12	—
324	1	—									14–20	22	—
325	Apyrexia	1–3 blood negative									9–11	11	—
326	—										—	—	46 days
327	—	2–15									5–11	11	—
328	2	1–3									9–15	15	—
329	1	1–5									18–24	37	—
330	Apyrexia	— blood negative									26–32	33	—
331	—										11–12	12	—
332	1	1–3									7–12	16	—
333	—	2–3									5–11	15	—
334	Apyrexia	1–16									—	—	25 days
335	2	1–5 blood negative									4–9	12	—
336	Apyrexia										7–13	14	—

GRAINS 30 FOR 3 WEEKS + 45 FOR 1 WEEK SERIES
(Cases 337-358)

These twenty-two cases were treated according to the method laid down in Provisional Instructions 24/Gen. No. 5,500 (A.M.D. 2) of April, 1917, issued by the War Office.

The Instructions were as follows:—

(*a*) Each patient is to be given 10-grain doses either of quinine sulphate, quinine hydrochloride, or quinine hydrobromide, in solution by the mouth, preferably before meals or at bedtime, at least three times daily, for the whole three weeks, this minimum dosage amounting to 30 grains of any of these salts a day. If, however, the Medical Officer thinks that the patient can take increased doses, four 10-grain doses may be administered during the second week of treatment, and 45 or 50 grains a day may be administered during the third week of treatment. The quinine treatment is then to be stopped entirely, and the patient may, if otherwise fit, be discharged to duty.

* * * * *

(*c*) The bowels should be regulated at the beginning of the course and during its continuance.

(*d*) The patient should be kept entirely in bed during the first three days of the course; should be allowed up only in the evenings during the remainder of the first week; should be allowed up only in the afternoons and evenings during the second week of the treatment; and may be allowed out of bed during the whole of the third week, but should not be permitted to go out of doors during the whole of the course.

(*e*) The diet should be fairly generous except when the patient is actually suffering from fever, and beer or wine may be allowed at the mid-day meal.

The amount of quinine given daily in our cases was grains 30 for three weeks and grains 45 for one week. Otherwise the treatment was, so far as practicable, precisely as laid down in the instructions.

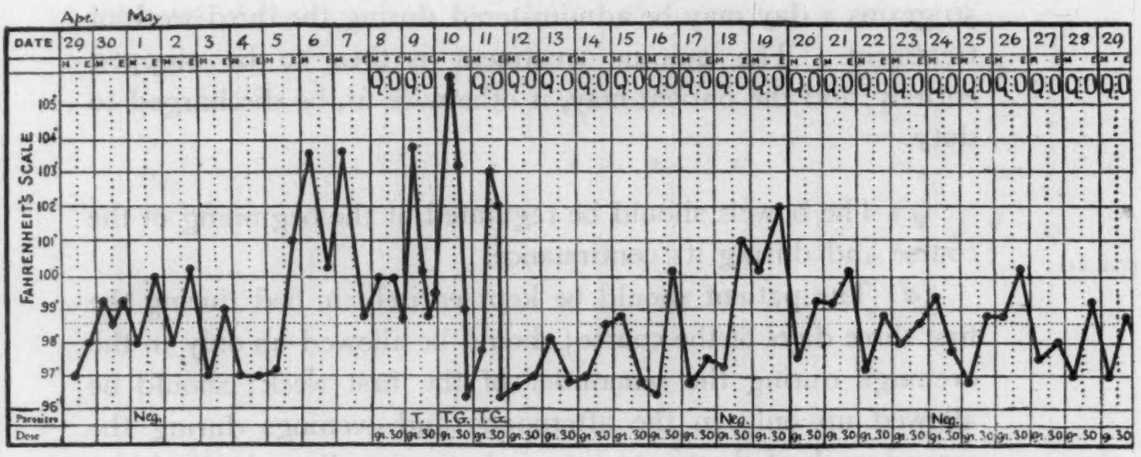
The temperature fell to normal in 1-4 days. Although all cases had parasites in the blood on, or a few days before, the first day of treatment, subsequent examinations were too infrequent to enable one to determine the exact day of disappearance.

Relapses.

During treatment. The average number of cases which had parasitic febrile relapses each week over a period of 4 weeks was 2·25 per cent. of cases treated, and that of all febrile cases (parasitic and non-parasitic) was 5·7 per cent. (Table VIII). The chart (Case 352) is a record of a case in which the temperature was not controlled by the treatment.

After treatment. Seventeen of twenty-two cases relapsed. Parasites reappeared in 1-58 days after the cessation of treatment, and febrile relapses occurred in 1-58 days. It should be noted that in one of the cases that did not relapse the observation period was less than 60 days, viz., 33 days (Table VI).

CASE 352 (Part I)



CASE 352 (Part II)

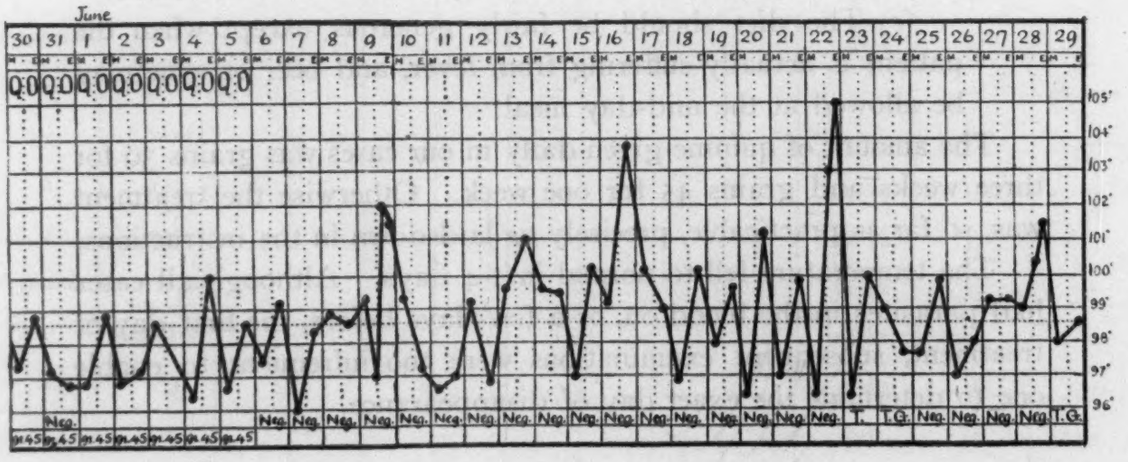


TABLE VI.

Results of oral administration of quinine sulphate in solution, grains 30 daily for 3 weeks, grains 45 daily for 1 week.

Number of Case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Number of febrile paroxysms (parasitic and non-parasitic) and results of blood examinations during treatment				Parasitic relapse occurred in — days after cessation of treatment	Febrile relapse (above 100° F.) occurred in — days after cessation of treatment	Observation period in cases which did not relapse
			Week of Treatment						
			1st	2nd	3rd	4th			
337	Apyrexia	—	0	0	0	0	—	—	33 days
338	1	—	0	0	0	0	39	39	—
339	1	—	0	0	0	**1**	1	1	—
340	Apyrexia	—	0	0	0	0	16	16	—
341	1	—	0	0	0	0	—	—	62 days
342	Apyrexia	—	0	0	0	0	16	15	—
343	Apyrexia	—	0	0	0	0	15	16	—
344	Apyrexia	—	0	0	0	0	—	—	62 days
345	Same day	—	0	0	0	0	1	1	—
346	1	—	0	**3**	0	0	9	9	—
347	2	—	0	0	0	0	15	17	—
348	Apyrexia	—	0	0	0	0	15	22	—
349	2	—	0	0	0	0	58	58	—
350	Same day	—	0	0	0	0	—	—	62 days
351	Apyrexia	—	0	0	0	0	16	18	—
352	4	—	0	**4***	**1***	**1***	4	4	—
353	1	—	0	0	0	0	15	16	—
354	1	—	0	0	0	0	10	14	—
355	Same day	—	0	0	0	0	8	9	—
356	Apyrexia	—	0	0	0	0	10	6	—
357	Apyrexia	—	0	0	0	0	—	—	100 days
358	Apyrexia	—	0	0	0	0	8	9	—

TABLE VII.

Summary of TABLE VI.

Grains 30 for 3 weeks + Grains 45 for 1 week series.

Week of Treatment	1st	2nd	3rd	4th
Number of cases treated	**22**	**22**	**22**	**22**
Number of cases having parasitic febrile relapses	0	1	0	1
Number of cases having non-parasitic febrile attacks	0	1	1	1
Number of cases having febrile paroxysms not examined	0	0	0	0
Grand total of all febrile cases	**0**	**2**	**1**	**2**
Total number of parasitic febrile relapses	0	3	0	1
Total number of non-parasitic febrile attacks	0	4	1	1
Total number of febrile paroxysms not examined	0	0	0	0
Grand total of all febrile paroxysms	**1**	**7**	**1**	**2**

TABLE VIII.

Analysis of TABLE VII.

Grains 30 for 3 weeks + Grains 45 for 1 week series.

Week of Treatment	1st	2nd	3rd	4th	Average per week
Percentage of parasitic febrile relapse cases per cases treated	0	4·5	0	4·5	**2·25**
Number of parasitic febrile relapses per parasitic febrile relapse case	0	3·0	0	1·0	**1·25**
Percentage of all febrile (parasitic and non-parasitic) cases per cases treated	0	9·1	4·5	9·1	**5·7**
Number of all febrile (parasitic and non-parasitic) paroxysms per febrile case	0	3·5	1·0	1·0	**1·4**

GRAINS 45 SERIES (Cases 359-377)

In these nineteen cases the temperature fell to normal on the day of, or in 1-2 days after, the commencement of treatment. Parasites disappeared from the cutaneous blood in 1-3 days.

Relapses.

During treatment. The average number of cases which had parasitic relapses each week, over a period of 8 weeks, was 1·8 per cent. of cases treated, and that of all febrile cases (parasitic and non-parasitic) was 10·0 per cent. (Table X). The number of cases on which this figure is based was 19 in the first week and 7 in the eighth week.

After treatment. Seven of nineteen cases relapsed. Parasites reappeared in 4-37 days after the cessation of treatment, and febrile relapses occurred in 10-37 days.

It should be noted that in three of the cases that did not relapse, the observation period was less than 60 days, viz., 28, 33, and 34 days, respectively (Table IX).

TABLE IX.

Results of oral administration of quinine sulphate in solution, grains 45, daily for 3–8 weeks.

Number of Case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Number of febrile paroxysms (parasitic and non-parasitic) and results of blood examinations during treatment								Parasitic relapse occurred in — days after cessation of treatment	Febrile relapse (above 100° F.) occurred in — days after cessation of treatment	Observation period in cases which did not relapse
			Week of Treatment										
			1st	2nd	3rd	4th	5th	6th	7th	8th			
359	2	2	1*	0	0	...	...	...	...	...	—	—	62 days
360	1	2	0	0	1*	...	...	...	...	...	14–20	10	—
361	1	3	0	0	0	...	...	...	...	...	—	—	63 days
362	1	2	0	0	0	0	0	...	...	...	10–13	14	—
363	Same day	1–2	0	0	0	1*	0	...	...	...	—	—	34 days
364	1	1–3	0	0	0	0	0	0	...	...	—	—	65 days
365	1	1–3	0	0	0	0	0	0	...	...	4–10	12	—
366	1	1	0	0	0	0	0	0	...	...	—	—	72 days
367	1	3	0	0	0	P	0	0	...	...	—	—	82 days
368	1	1–3	0	1*	0	0	0	0	...	...	37	37	—
369	1	2	0	0	0	0	0	0	0	...	—	—	60 days
370	1	3	0	2*	0	0	0	0	0	...	11–14	14	—
371	1	2	0	0	0	0	1*	0	0	0	—	—	28 days
372	Same day	2	0	0	0	0	0	0	0	0	14	14	—
373	1	2	0	0	0	0	0	0	0	1	—	—	60 days
374	Same day	2	0	0	0	0	0	0	0	0	—	—	60 days
375	1	2	0	0	0	0	0	1*	0	1*	26	26	—
376	2	2	0	0	0	0	0	0	0	0	—	—	60 days
377	1	2	0	1*	2*	0	0	0	0	0	—	—	33 days

TABLE X.

Summary of TABLE IX.

Grains 45 series.

Week of Treatment	1st	2nd	3rd	4th	5th	6th	7th	8th
Number of cases treated ...	**19**	**19**	**19**	**16**	**16**	**14**	**9**	**7**
Number of cases having parasitic febrile relapses	0	0	0	0	0	0	0	1
Number of cases having non-parasitic febrile attacks	1	3	2	1	1	1	0	1
Number of cases having febrile paroxysms not examined	0	0	0	0	0	0	0	0
Grand total of all febrile cases	**1**	**3**	**2**	**1**	**1**	**1**	**0**	**2**
Total number of parasitic febrile relapses	0	0	0	0	0	0	0	1
Total number of non-parasitic febrile attacks	1	4	3	1	1	1	0	1
Total number of febrile paroxysms not examined	0	0	0	0	0	0	0	0
Grand total of all febrile paroxysms	**1**	**4**	**3**	**1**	**1**	**1**	**0**	**2**

TABLE XI.

Analysis of TABLE X.

Grains 45 series.

Week of Treatment	1st	2nd	3rd	4th	5th	6th	7th	8th	Average per week
Percentage of parasitic febrile relapse cases per cases treated	0	0	0	0	0	0	0	14·3	**1·8**
Number of parasitic febrile relapses per parasitic febrile relapse case	0	0	0	0	0	0	0	1·0	**0·1**
Percentage of all febrile (parasitic and non-parasitic) cases per cases treated ...	5·3	15·8	10·5	6·2	6·2	7·1	0	28·6	**10·0**
Number of all febrile (parasitic and non-parasitic) paroxysms per febrile case ...	1·0	1·3	1·5	1·0	1·0	1·0	0	1·0	**1·0**

COMPARISON OF RESULTS OBTAINED FROM THE VARIOUS TREATMENTS

In Table XII, the palliative values of the treatments, Grains 30 and Grains 45 respectively, are compared. The Grains 30 for three weeks + Grains 45 for one week is omitted, as the treatment extended over four weeks only, and is therefore not comparable. It will be seen that as a palliative treatment the larger dose is more efficient than the smaller.

TABLE XII.

Comparison of palliative results obtained from the different treatments.

	Grains 30	Grains 45
Percentage of parasitic febrile relapse cases per cases treated (average per week)	7·2	1·8
Number of parasitic febrile relapses per parasitic febrile relapse case (average per week) ...	0·7	0·1
Percentage of all febrile (parasitic and non-parasitic) cases per cases treated (average per week) ...	17·3	10·0
Number of all febrile (parasitic and non-parasitic) paroxysms per febrile case (average per week)	1 0	1·0

In Table XIII the curative results obtained in all the series are compared.

As stated in previous studies, it was our aim to observe all cases treated for a period of at least 60 days after cessation of treatment. This is of course a purely arbitrary period determined by conditions over which we had no control. Had the cases always been observed for this period it would be possible to compare the results of the various treatments employed, the information obtained from any of the treatments reading as follows:—Number of cases treated x, number of cases which relapsed in an observation period of 60 days y, percentage of cases which relapsed z.

It is obvious that if certain cases were observed for only 20 days after cessation of treatment whilst others were observed for 60 days, the results cannot be compared. Consequently in considering the

percentage of relapses observed after the various forms of prolonged (*continuous*) treatment adopted by us it is necessary, as unfortunately some of the cases were not observed for the full period of 60 days after treatment, to give two sets of figures:—(1) Minimum figure, i.e., the percentage actually observed to have relapsed; (2) Maximum figure, i.e. one based on the assumption that all cases not observed for a period of 60 days relapsed before the expiration of that period. It is clear that the true percentage of cases which would relapse in an observation period of 60 days must lie somewhere between these two extremes, probably for reasons which we shall consider in a future paper, much nearer the minimum than the maximum value.

The superiority of the Grains 45 treatment is clear, as the maximum percentage of relapses possible in an observation period of 60 days is less than the observed minimum values in any of the other series (Table XIII).

TABLE XIII.

Comparison of curative results obtained from the different treatments.

Dose in grains	Duration of treatment	Number of cases treated	Number of cases which relapsed	Number of cases not relapsing, but observed for less than 60 days	Percentage of cases which relapsed	
					minimum	maximum
20	14–15 weeks	5	3	1	60·0	80·0
30	5–18 weeks	14	10	0	71·0	71·0
30	8 weeks (camp)	29	24	5	79·3	100·0
30	3 weeks	22	17	1	77·4	81·8
45	1 week					
45	3–8 weeks	19	7	3	36·8	52·6

TOLERANCE OF THE TREATMENTS

Practically all the cases were able to take a daily dose of grains 20-30 for eight weeks or more. When, however, the daily dose reached grains 45 it was found that only seven of nineteen cases were able to complete the full eight weeks' treatment. In the remaining twelve the treatment had to be stopped prematurely owing to tremors and vomiting.

CONCLUSION

Of the various forms of *continuous* treatments used by us, that of Grains 45 is the best.

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STUDIES IN THE TREATMENT OF MALARIA

VIII. ORAL ADMINISTRATION OF QUININE SULPHATE FOR TWO CONSECUTIVE DAYS WEEKLY OVER PROLONGED PERIODS, IN SIMPLE TERTIAN MALARIA

BY

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Interrupted treatment, i.e. treatment on one or more days at intervals, instead of daily, over a given period has been recommended by various authors.

Laveran (1907) advocates this mode of treatment on the ground that 'si l'on prescrit 1 gr. de chlorhydrate de quinine en une dose, le sang se trouve à un moment donné plus chargé de quinine, et par suite plus toxique pour les hématozoaires, que si, pendant quatre jours, on prescrivait 0 gr. 25 de chlorhydrate de quinine.'

Thomson, D. (1917), on the other hand, is of opinion that 'The most important point of all is to begin treatment early and to avoid the intermittent use of quinine. Do not play with the parasites by half killing them with the drug and then allowing them a few days' respite to recover from the poison.' He brings forward no evidence to support the view that parasites can be half killed by any dose of quinine, and recover from the process.

In No. VI of these Studies (1918) we have recorded the effects on parasites of quinine sulphate, administered on two days only: how they disappear from the cutaneous blood and how after a

certain interval they reappear. We consequently proceeded to observe what would happen if before the expected time of reappearance quinine was administered on two additional days, i.e. in effect to observe the result of an interrupted treatment.

The treatments were given on two consecutive days each week, e.g. Saturday and Sunday, and were extended over a period of eight weeks or more.

All the cases were adult males infected in Macedonia at least six months previously, and all had had more or less quinine during this period. In every instance a diagnosis of simple tertian malaria was made by microscopic examination. In the vast majority parasites were present in the blood on the day treatment commenced. In the remainder, although a blood examination was not made on the first day of treatment, parasites were present 2-3 days previously.

As in No. VII of these Studies (1918), the results obtained are considered (1) from the palliative point of view and (2) from the curative point of view; and hence there is a definite basis for comparison of the two methods, namely, *continuous* and *interrupted* quinine treatments.

So far as possible, blood examinations were made once weekly and also whenever the temperature reached 100° F. or over, which in this, as in all our previous papers, is regarded as a febrile paroxysm.

In the following charts and tables:—

Numerals 0, 1, 2, etc. = number of parasitic febrile relapses weekly.
Numerals 1*, 2*, etc. = number of non-parasitic febrile attacks weekly.
Numerals 1°, 2°, etc. = number of febrile paroxysms not examined microscopically, weekly.
P. = non-febrile parasitic relapse.
Q.O. = quinine sulphate orally.
T. = simple tertian trophozoites or schizonts.
G. = simple tertian gametes.
cr. = malignant tertian gametes.
Neg. = no parasites found.

Note.—A rise of temperature above 100° F., of which the nature is unknown, is termed a *febrile attack*. A similar rise of temperature accompanied by parasites in the blood at the time, or within three days, is termed a *parasitic febrile relapse* or *true relapse*. The term *paroxysm* is used indifferently to denote any febrile disturbance of 100° F. or more.

As it was not found possible to retain all cases treated in hospital for eight weeks, the numbers in some of the series diminished from week to week: for example, in Table VIII the experiment commenced

with 203 cases, but at the end of the eighth week only 92 remained in hospital. In order, therefore, that the results obtained in the various series of experiments may have a comparative value it is necessary to express the cases having true relapses or febrile attacks as percentages of the total cases undergoing treatment in any particular week. This has been done in Tables III, VI, IX and XII, in which the results given in Tables II, V, VIII and XI are analysed. By this means the following sets of figures, each having a comparative value, are obtained:—

1. The number of cases which had parasitic febrile relapses each week, expressed as percentages of all cases treated.

2. The number of parasitic febrile relapses experienced per week by each parasitic febrile relapse case.

3. The number of cases which had febrile paroxysms (parasitic and non-parasitic) each week, expressed as percentages of all cases treated.

4. The number of febrile paroxysms (parasitic and non-parasitic) experienced per week by each febrile (parasitic and non-parasitic) case.

The duration of treatment in each case is clearly shown in the tables.

GRAINS 10 SERIES (Cases 378-395)

In these eighteen cases the temperature fell to normal on the day of, or 1-2 days after, the commencement of treatment. In fifteen cases parasites disappeared from the cutaneous blood in 1-3 days, and in three cases the examinations were too infrequent to give a precise figure.

Relapses.

During treatment. The average number of cases which had parasitic febrile relapses each week, over a period of 8 weeks, was 6·3 per cent. of all cases treated, and that of all febrile cases (parasitic and non-parasitic) was 10·4 per cent (Table III). The number of cases on which these figures are based is, however, small (eighteen).

After treatment. Seven of the fifteen cases observed for 30 days or more after cessation of treatment relapsed. Parasites reappeared in 1-15 days, and febrile relapses occurred in 1-15 days after cessation of treatment. In all cases which did not relapse the observation period was less than 60 days (Table I).

TABLE I.

Results of oral administration of quinine sulphate in solution, grains 10, on two consecutive days weekly for 8 to 16 weeks.

Number of Case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Number of febrile paroxysms (parasitic and non-parasitic) and results of blood examinations during treatment — Week of treatment															Parasitic relapse occurred in — days after cessation of treatment	Febrile relapse (above 100° F.) occurred in — days after cessation of treatment	Observation period in cases which did not relapse
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th			
378	same day	1	0	0	0	P	0	0	0	0	...	...	...	...	...	...	...	...	—	—	54 days
379	2	3	0	0	0	0	1*	0	0	0	...	...	...	...	...	...	...	...	13	13	—
380	1	2	0	0	1	1	0	cr.	0	0	...	...	...	...	...	...	...	...	—	—	43 days
381	1	2	0	0	0	0	0	0	0	0	...	...	...	...	...	...	...	...	15	13–15	—
382	same day	2	0	0	0	0	0	0	0	0	...	...	...	...	...	...	...	...	—	—	41 days
383	1	1	0	0	0	0	0	0	0	0	...	...	...	...	...	...	...	...	—	—	42 days
384	1	2	0	0	2	0	0	0	0	0	...	...	...	...	...	...	...	...	12	15	—
385	2	2	0	0	0	0	1*	0	0	0	...	...	...	...	...	...	...	...	—	—	47 days
386	2	3–4	0	0	0	0	0	0	0	0	...	...	...	...	...	...	...	...	5	5	—
387	1	3	0	0	0	0	0	0	0	0	...	...	...	...	...	...	...	...	—	—	16 days
388	1	3–9	0	0	0	0	1*	0	0	0	1*	...	...	...	...	...	...	...	—	—	32 days
389	2	3–9	0	0	0	0	1*	0	0	0	0	...	...	...	...	...	...	...	—	—	30 days
390	1	2	0	0	0	0	0	1*	0	0	0	...	...	...	...	...	...	...	—	—	43 days
391	1	3	0	0	0	0	0	0	0	0	0	...	...	...	...	...	...	...	7	7	—
392	1	1–2	0	0	0	0	0	0	0	0	0	0	...	...	...	...	...	...	—	—	8 days
393	2	2	3	0	0	0	3	0	1	3	0	2	...	...	...	...	...	...	9–11	11	—
394	same day	1	0	0	0	0	0	0	0	0	0	0	0	...	...	...	...	...	—	—	not observed
395	2	2	0	0	0	0	3*	0	1	3	0	1	0	0	3	0	0	2	1	1	—

TABLE II.

Summary of Table I.

Grains 10 series.

Week of treatment	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Number of cases treated ...	**18**	**18**	**18**	**18**	**18**	**18**	**18**	**18**	**8**	**4**	**2**	**1**	**1**	**1**	**1**	**1**
Number of cases having parasitic febrile relapses	1	0	2	1	1	0	2	2	0	2	0	0	1	0	0	1
Number of cases having non-parasitic febrile attacks	0	0	0	0	5	1	0	0	1	0	0	0	0	0	0	0
Number of cases having febrile paroxysms not examined	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Grand total of all febrile cases ...	**1**	**0**	**2**	**1**	**6**	**1**	**2**	**2**	**1**	**2**	**0**	**0**	**1**	**0**	**0**	**1**
Total number of parasitic febrile relapses	3	0	3	1	3	0	2	6	0	3	0	0	3	0	0	2
Total number of non-parasitic febrile attacks	0	0	0	0	7	1	0	0	1	0	0	0	0	0	0	0
Total number of febrile paroxysms not examined	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Grand total of all febrile paroxysms	**3**	**0**	**3**	**1**	**10**	**1**	**2**	**6**	**1**	**3**	**0**	**0**	**3**	**0**	**0**	**2**

TABLE III.

Analysis of TABLE II, giving the results for the first 8 weeks.

Grains 10 series.

Week of treatment	1st	2nd	3rd	4th	5th	6th	7th	8th	Average per week
Percentage of parasitic febrile relapse cases per cases treated	5·6	0	11·1	5·6	5·6	0	11·1	11·1	**6·3**
Number of parasitic febrile relapses per parasitic febrile relapse case	3·0	0	1·5	1·0	3·0	0	1·0	3·0	**1·6**
Percentage of all febrile (parasitic and non-parasitic) cases per cases treated	5·6	0	11·1	5·6	33·3	5·6	11·1	11·1	**10·4**
Number of all febrile (parasitic and non-parasitic) paroxysms per febrile case ...	3·0	0	1·5	1·0	1·7	1·0	1·0	3·0	**1·5**

As examples of how the figures in Table III are obtained we may take 5.6 in the first column of the first line; this signifies that in the first week among eighteen cases under treatment (Table II) there was one parasitic febrile relapse case, giving a percentage of 5·6: again, 33·3 in the fifth column of the third line signifies that in the fifth week among eighteen cases under treatment (Table II) there were six febrile cases, giving a percentage of 33·3.

GRAINS 15 SERIES (Cases 396-459)

In these sixty-four cases the temperature fell to normal on the day of, or 1-2 days after, the commencement of treatment. In twenty-four cases parasites disappeared from the cutaneous blood in 1-3 days, in seven cases examinations were too infrequent to give a precise figure, and in thirty-three there were no records (Table IV).

Relapses.

During treatment. The average number of cases which had parasitic febrile relapses each week, over a period of 8 weeks, was 18·8 per cent. of all cases treated, and that of all febrile cases (parasitic and non-parasitic) was 20·1 per cent. (Table VI). The number of cases on which these figures are based is sixty-four in the first week and fifty-six in the eighth week.

After treatment. The cases were not observed after cessation of treatment.

TABLE IV.

Results of oral administration of quinine sulphate in solution, grains 15, on two consecutive days weekly for 2–11 weeks.

Number of Case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Number of febrile paroxysms (parasitic and non-parasitic) and results of blood examinations during treatment										
			Week of Treatment										
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th
396	2	—	**2**	**3**	...	...	...	...	...	...	...	...	...
397	Same day	—	0	0	...	...	...	...	...	...	...	...	...
398	1	1–2	0	0	0	0	...	...	...	...	...	...	...
399	Same day	—	0	0	0	0	0	0	...	...	...	...	...
400	2	—	0	0	0	0	0	0	...	...	...	...	...
401	1	—	0	0	0	0	0	0	0	...	...	...	...
402	2	—	0	0	**1**	0	0	0	0	...	...	...	...
403	1	—	0	0	0	0	0	0	0	...	...	...	...
404	—	—	**2**	**2**	**4**	**2**	**4**	**3**	**2**	**1**	...	...	...
405	1	1	0	0	0	0	0	0	0	0	...	...	...
406	1	2	0	0	0	0	0	0	0	0	...	...	...
407	1	2	0	0	0	0	0	**1***	0	**1***	...	...	...
408	2	2	0	0	0	0	0	0	0	0	...	...	...
409	2	2	0	0	0	0	0	0	0	0	...	...	...
410	1	2	0	0	0	0	0	0	0	0	...	...	...
411	1	3–4	0	0	0	0	**1**	**1**	0	**3**	...	...	...
412	2	2	0	0	0	0	0	0	0	0	...	...	...
413	1	1–6	0	0	0	0	**3**	0	0	0	...	...	...
414	Same day	2	0	0	0	0	0	0	0	0	...	...	...
415	2	2	0	**1**	**1**	**1**	**4**	**1**	0	**1**	...	...	...
416	1	2	0	0	0	0	0	0	0	0	...	...	...
417	2	2	0	0	0	0	0	0	0	0	...	...	...

TABLE IV—*continued*.

Results of oral administration of quinine sulphate in solution, grains 15, on two consecutive days weekly for 2-11 weeks.

Number of Case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Number of febrile paroxysms (parasitic and non-parasitic) and results of blood examinations during treatment										
			Week of Treatment										
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th
418	1	3	0	0	0	0	0	0	0	0	...	...	...
419	Same day	1	0	0	0	**1**	**2**	0	0	**1**	...	...	...
420	1	3	0	0	0	0	0	0	0	0	...	...	...
421	1	2	0	0	0	0	0	0	0	0	...	...	...
422	1	1–6	0	0	**2**	0	**2**	0	0	0	...	...	...
423	1	1–6	0	**2**	**1**	0	0	0	0	0	...	...	...
424	2	2	0	0	0	0	0	0	0	**2**	...	...	...
425	1	—	0	**2**	0	0	0	**2**	0	0	0	...	...
426	2	3–4	0	0	0	0	0	0	0	0	0	...	...
427	1	—	0	0	**4**	0	0	**2**	0	0	**1**	...	...
428	1	—	0	0	0	0	0	0	**1**	0	0	...	...
429	1	—	0	0	0	0	**1**	0	0	0	**1**	...	...
430	Apyrexia	—	0	0	0	0	0	0	**1**	0	**1**	...	...
431	1	—	0	0	**1**	**2**	0	0	0	0	**2**	...	...
432	1	—	0	**2**	**P**	0	0	**2**	0	0	0	...	...
433	1	2	0	0	**1**	0	0	0	0	0	0	...	...
434	1	—	**3**	**3**	**4**	**1**	**3**	...	**3**	**3**	**1**	...	...
435	1	3	0	0	0	**1***	0	0	0	0	0	...	...
436	Same day	1	0	0	0	0	0	0	0	0	0	...	...
437	Apyrexia	2–8	0	0	0	0	0	0	0	0	0	...	...
438	1	1	0	0	**1**	0	0	0	**1**	0	0	...	...
439	Apyrexia	2	0	**1**	0	0	**1**	0	0	0	**1**	...	...

TABLE IV—*continued.*

Results of oral administration of quinine sulphate in solution, grains 15, on two consecutive days weekly for 2-11 weeks.

Number of Case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Number of febrile paroxysms (parasitic and non-parasitic) and results of blood examinations during treatment										
			Week of Treatment										
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th
440	Apyrexia	—	1°	0	0	2	1	1	2	0	0	0	...
441	2	1–4	0	0	0	0	1	0	3	0	0	1	...
442	Apyrexia	—	0	5	6	3	0	0	0	0	0	0	...
443	1	—	0	0	0	0	0	0	0	0	0	0	...
444	1	—	0	0	0	0	0	0	0	0	0	0	...
445	1	—	0	0	0	0	0	2	2	0	0	0	...
446	2	2	0	0	0	4	0	0	0	3	0	0	...
447	2	—	0	0	0	0	0	0	0	0	0	0	...
448	1	—	0	0	0	2	1	0	0	0	0	0	...
449	—	—	3	2	0	0	0	0	2	0	0	0	...
450	Apyrexia	—	0	0	0	0	0	0	0	0	0	0	0
451	1	—	0	0	0	0	4	0	2	1	0	0	0
452	1	—	0	0	0	0	0	0	0	0	0	0	0
453	1	—	0	0	0	0	0	0	0	0	0	0	0
454	1	1	0	1	0	0	2	1	0	3	0	1	1
455	2	—	1	0	0	0	0	0	0	0	0	0	0
456	2	—	0	0	0	0	0	0	0	0	0	0	0
457	Apyrexia	—	1°	1°	3	1	1	1	1	0	2	1	0
458	1	—	0	0	1	1	1	0	0	0	0	0	0
459	Apyrexia	—	2	1	0	2	1	2	3	0	1°	2°	0

TABLE V.

Summary of TABLE IV.

Grains 15 series.

Week of treatment ...	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th
Number of cases treated	**64**	**64**	**62**	**62**	**61**	**61**	**59**	**56**	**35**	**20**	**10**
Number of cases having parasitic febrile relapses	6	12	13	12	17	11	12	9	7	3	1
Number of cases having non-parasitic febrile attacks	0	0	0	1	0	1	0	1	0	0	0
Number of cases having febrile paroxysms not examined	2	1	0	0	0	0	0	0	1	1	0
Grand total of all febrile cases ...	**8**	**13**	**13**	**13**	**17**	**12**	**12**	**10**	**8**	**4**	**1**
Total number of parasitic febrile relapses ...	13	25	30	22	33	18	23	18	9	3	1
Total number of non-parasitic febrile attacks	0	0	0	1	0	1	0	1	0	0	0
Total number of febrile paroxysms not examined	2	1	0	0	0	0	0	0	1	2	0
Grand total of all febrile paroxysms	**15**	**26**	**30**	**23**	**33**	**19**	**23**	**19**	**10**	**5**	**1**

TABLE VI.

Analysis of TABLE V, giving the results for the first 8 weeks.

Grains 15 series.

Week of treatment	1st	2nd	3rd	4th	5th	6th	7th	8th	Average per week
Percentage of parasitic febrile relapse cases per cases treated	9·4	18·7	21·0	19·4	27·8	18·0	20·4	16·1	**18·8**
Number of parasitic febrile relapses per parasitic febrile relapse case	2·2	2·1	2·3	1·8	1·9	1·6	1·9	2·0	**2·0**
Percentage of all febrile (parasitic and non-parasitic) cases per cases treated ...	12·5	20·3	21·0	21·0	27·8	20·0	20·4	18·0	**20·1**
Number of all febrile (parasitic and non-parasitic) paroxysms per febrile case ...	1·9	2·0	2·3	1·8	1·9	1·6	1·9	1·9	**1·9**

GRAINS 30 SERIES (Cases 460-662)

In these two hundred and three cases the temperature fell to normal on the day of, or 1-5 days after, the commencement of treatment. In twenty-four cases parasites disappeared from the cutaneous blood in 1-3 days, in thirteen cases examinations were too infrequent to give a precise figure, and in 166 cases the time of disappearance was not recorded (Table VII).

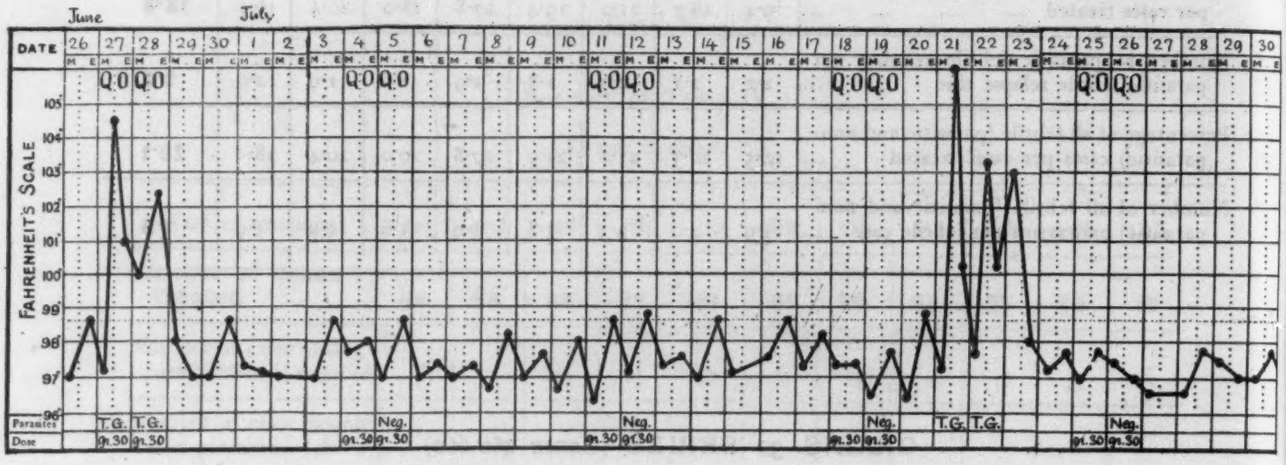
Relapses.

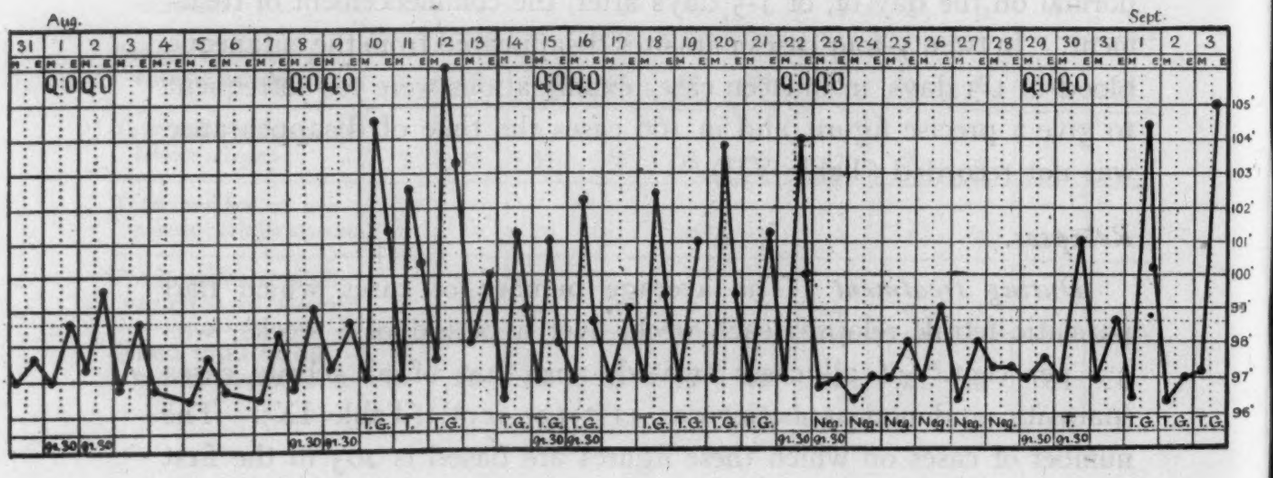
During treatment. The average number of cases which had parasitic febrile relapses each week, over a period of 8 weeks, was 3·2 per cent.* of all cases treated, and that of all febrile cases (parasitic and non-parasitic) was 11·3 per cent. (Table IX). The number of cases on which these figures are based is 203 in the first week and 92 in the eighth week.

After treatment. The cases were not observed after cessation of treatment.

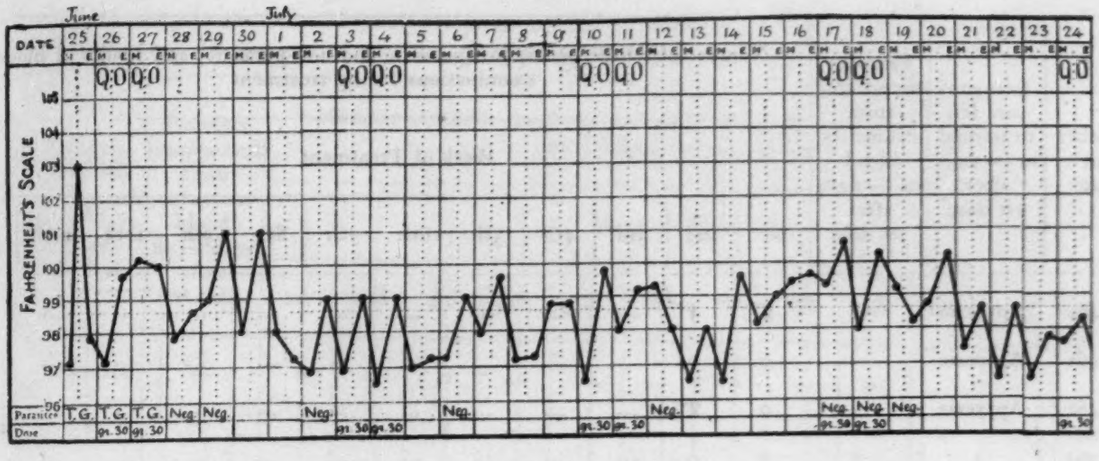
*This figure is undoubtedly too low, as in many of the febrile paroxysms the blood was not examined (vide Table VII).

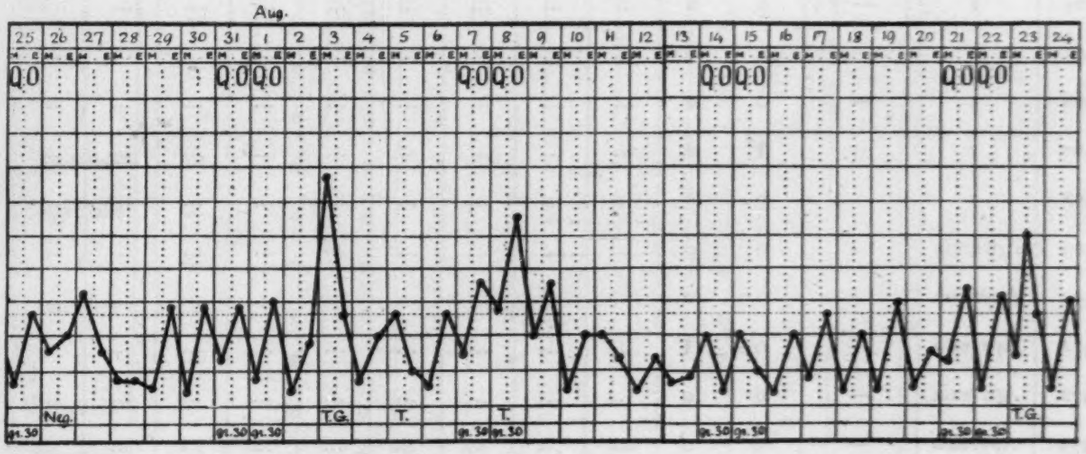
Case 633


June
July
DATE
26 27 28 29 30 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
FAHRENHEIT'S SCALE
105 104 103 102 101 100 99 98 97 96
Q O
Paraules
Dose
T. G. gr. 30
Neg. gr. 30


Aug.
Sept.
31 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 1 2 3
Q O
105 104 103 102 101 100 99 98 97 96
T. G.
T.
Neg.
gr. 30

Case 662


June
July
DATE
FAHRENHEIT'S SCALE
Parasites
Dose
T.G.
Neg.
gr. 30


Aug.
Neg.
T.G.
T.
gr. 30

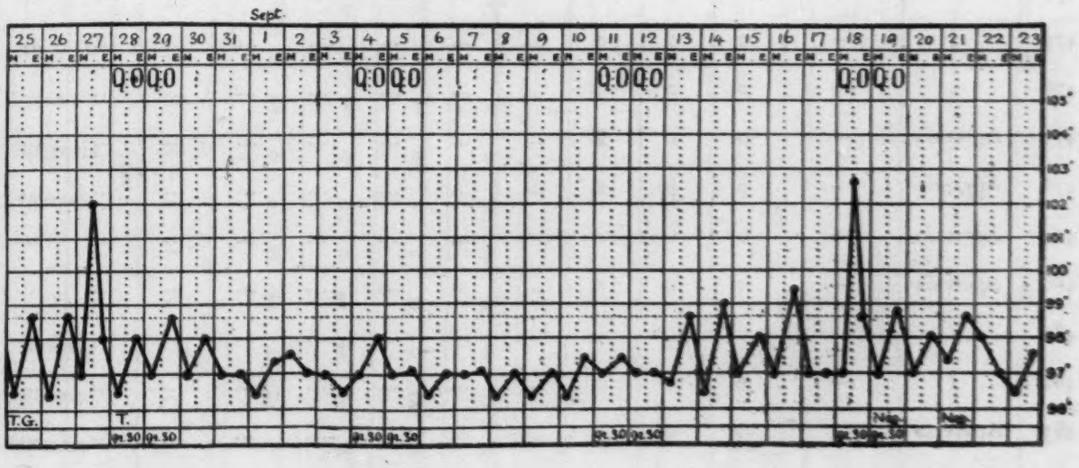

Sept.
T.G.
T.
Neg.
gr. 30

TABLE VII.

Results of oral administration of quinine sulphate in solution, grains 30, on two consecutive days weekly for 2–12 weeks.

Number of Case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Number of febrile paroxysms (parasitic and non-parasitic) and results of blood examinations during treatment											
			Week of Treatment											
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
460	Apyrexia	—	2°	1°	...	...	...	...	...	...	...	...	...	...
461	Apyrexia	—	0	0	...	...	...	...	...	...	...	...	...	...
462	Apyrexia	—	0	2°	...	...	...	...	...	...	...	...	...	...
463	1	—	0	0	...	...	...	...	...	...	...	...	...	...
464	1	—	0	0	...	...	...	...	...	...	...	...	...	...
465	3	—	0	0	...	...	...	...	...	...	...	...	...	...
466	1	—	0	0	...	...	...	...	...	...	...	...	...	...
467	1	—	1°	0	...	...	...	...	...	...	...	...	...	...
468	1	—	0	0	...	...	...	...	...	...	...	...	...	...
469	Apyrexia	—	0	0	...	...	...	...	...	...	...	...	...	...
470	Apyrexia	—	0	0	...	...	...	...	...	...	...	...	...	...
471	Apyrexia	—	0	1°	...	...	...	...	...	...	...	...	...	...
472	Apyrexia	—	0	0	...	...	...	...	...	...	...	...	...	...
473	Apyrexia	—	0	0	...	...	...	...	...	...	...	...	...	...
474	1	—	0	0	0	...	...	...	...	...	...	...	...	...
475	3	—	0	0	0	...	...	...	...	...	...	...	...	...
476	1	—	0	0	0	...	...	...	...	...	...	...	...	...
477	Apyrexia	—	0	0	2°	...	...	...	...	...	...	...	...	...
478	Apyrexia	—	0	0	0	...	...	...	...	...	...	...	...	...
479	Apyrexia	—	0	0	0	...	...	...	...	...	...	...	...	...
480	Apyrexia	—	0	0	0	...	...	...	...	...	...	...	...	...
481	1	—	0	0	0	...	...	...	...	...	...	...	...	...
482	1	—	0	0	0	...	...	...	...	...	...	...	...	...
483	Apyrexia	—	0	0	0	...	...	...	...	...	...	...	...	...
484	Apyrexia	—	0	0	0	...	...	...	...	...	...	...	...	...
485	Apyrexia	—	0	0	0	...	...	...	...	...	...	...	...	...

TABLE VII—*continued.*

Results of oral administration of quinine sulphate in solution, grains 30, on two consecutive days weekly for 2–12 weeks.

Number of Case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Number of febrile paroxysms (parasitic and non-parasitic) and results of blood examinations during treatment										
			Week of Treatment										
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
486	Apyrexia	—	0	0	0	...	...	...	...	...	...	...	...	...
487	4	—	1	0	2	...	...	...	...	...	...	...	...	...
488	1	—	0	0	0	1°	...	...	...	...	...	...	...	...
489	1	—	0	0	0	0	...	...	...	...	...	...	...	...
490	1	—	0	0	0	0	...	...	...	...	...	...	...	...
491	1	—	0	0	0	0	...	...	...	...	...	...	...	...
492	Apyrexia	—	0	0	0	0	...	...	...	...	...	...	...	...
493	Apyrexia	—	0	0	0	0	...	...	...	...	...	...	...	...
494	1	—	0	0	0	0	...	...	...	...	...	...	...	...
495	Apyrexia	—	0	0	0	1°	...	...	...	...	...	...	...	...
496	1	—	1°	0	0	1°	...	...	...	...	...	...	...	...
497	Apyrexia	—	0	0	0	0	...	...	...	...	...	...	...	...
498	Apyrexia	—	0	3	0	1*	...	...	...	...	...	...	...	...
499	2	—	0	0	0	1°	...	...	...	...	...	...	...	...
500	1	—	0	0	0	1°	...	...	...	...	...	...	...	...
501	1	—	1°	1°	1°	0	...	...	...	...	...	...	...	...
502	Apyrexia	—	0	0	0	0	...	...	...	...	...	...	...	...
503	Apyrexia	—	0	0	0	0	...	...	...	...	...	...	...	...
504	1	—	0	0	0	3	...	...	...	...	...	...	...	...
505	1	—	2°	2°	0	0	...	...	...	...	...	...	...	...
506	Apyrexia	—	0	1°	0	0	...	...	...	...	...	...	...	...
507	Apyrexia	—	2°	1°	2°	0	0	...	...	...	...	...	...	...
508	Apyrexia	—	0	0	0	0	0	...	...	...	...	...	...	...
509	1	—	0	0	0	0	0	...	...	...	...	...	...	...
510	Apyrexia	—	0	0	0	0	0	...	...	...	...	...	...	...
511	1	—	0	0	2°	0	0	...	...	...	...	...	...	...

TABLE VII—*continued.*

Results of oral administration of quinine sulphate in solution, grains 30, on two consecutive days weekly for 2–12 weeks.

Number of Case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Number of febrile paroxysms (parasitic and non-parasitic) and results of blood examinations during treatment											
			Week of Treatment											
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
512	1	—	0	0	0	0	0	...	...	...	...	...	...	...
513	Apyrexia	—	0	1°	0	1°	1°	...	...	...	...	...	...	...
514	1	—	0	0	0	0	0	...	...	...	...	...	...	...
515	Apyrexia	—	0	0	0	0	0	...	...	...	...	...	...	...
516	Apyrexia	—	0	0	0	0	0	...	...	...	...	...	...	...
517	1	—	0	0	0	0	0	...	...	...	...	...	...	...
518	Apyrexia	—	0	0	0	0	0	...	...	...	...	...	...	...
519	Apyrexia	—	0	0	0	0	0	...	...	...	...	...	...	...
520	1	—	0	0	0	0	0	...	...	...	...	...	...	...
521	Apyrexia	—	0	0	0	0	0	...	...	...	...	...	...	...
522	Apyrexia	—	0	0	0	0	0	...	...	...	...	...	...	...
523	Apyrexia	—	0	0	0	2*	0	...	...	...	...	...	...	...
524	Apyrexia	—	0	1°	0	0	1°	...	...	...	...	...	...	...
525	1	—	0	0	0	0	0	...	...	...	...	...	...	...
526	1	—	0	0	0	0	0	...	...	...	...	...	...	...
527	1	—	0	0	0	0	2°	...	...	...	...	...	...	...
528	1	—	0	0	0	0	0	0	...	...	...	...	...	...
529	1	—	0	0	0	0	0	0	...	...	...	...	...	...
530	Apyrexia	—	0	1*	0	0	0	0	...	...	...	...	...	...
531	3	—	1°	0	0	0	0	0	...	...	...	...	...	...
532	Apyrexia	—	0	0	0	0	0	0	...	...	...	...	...	...
533	1	—	0	0	0	0	0	0	...	...	...	...	...	...
534	1	—	0	0	0	0	0	0	...	...	...	...	...	...
535	1	—	0	0	0	2P	0	2°	...	...	...	...	...	...
536	Apyrexia	—	0	0	0	0	0	1°	...	...	...	...	...	...
537	Apyrexia	—	0	0	0	0	0	0	...	...	...	...	...	...

TABLE VII—*continued.*

Results of oral administration of quinine sulphate in solution, grains 30, on two consecutive days weekly for 2–12 weeks.

Number of Case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Number of febrile paroxysms (parasitic and non-parasitic) and results of blood examinations during treatment											
			Week of Treatment											
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
538	Apyrexia	—	0	0	0	0	0	0	...	...	...	...	...	...
539	Apyrexia	—	0	1°	0	1°	0	0	...	...	...	...	...	...
540	3	—	0	...	0	0	0	0	...	...	...	...	...	...
541	3	—	0	1°	2°	1°	0	0	...	...	...	...	...	...
542	Apyrexia	—	0	0	0	0	0	0	...	...	...	...	...	...
543	1	—	2°	0	1°	0	0	0	...	...	...	...	...	...
544	Apyrexia	—	0	0	0	0	0	0	...	...	...	...	...	...
545	Apyrexia	—	0	0	0	0	0	0	...	...	...	...	...	...
546	Apyrexia	1–7	0	0	0	0	0	0	...	...	...	...	...	...
547	Apyrexia	—	0	1°	0	0	0	0	0	...	...	...	...	...
548	1	1–2	0	0	2°	1°	0	0	0	...	...	...	...	...
549	Apyrexia	—	0	0	0	2	0	0	2°	...	...	...	...	...
550	Apyrexia	—	0	0	0	0	0	0	0	...	...	...	...	...
551	5	—	2°	0	0	2	2	2	2°	...	...	...	...	...
552	2	—	0	2	0	2	0	0	0	...	...	...	...	...
553	2	—	2	1	0	0	0	0	0	...	...	...	...	...
554	Apyrexia	—	0	0	0	0	0	0	0	...	...	...	...	...
555	Apyrexia	—	0	2°	1°	0	0	0	1°	...	...	...	...	...
556	2	—	0	0	0	0	0	0	0	...	...	...	...	...
557	2	—	0	0	0	1°	1°	0	0	...	...	...	...	...
558	Apyrexia	—	0	0	0	0	0	0	0	...	...	...	...	...
559	Apyrexia	—	0	0	0	0	0	0	0	...	...	...	...	...
560	2	—	0	0	1°	0	0	0	0	...	...	...	...	...
561	Apyrexia	—	3	0	0	0	0	0	0	...	...	...	...	...
562	Apyrexia	—	0	0	0	0	0	0	0	...	...	...	...	...
563	Apyrexia	—	2	0	0	2	0	0	0	...	...	...	...	...

TABLE VII—*continued.*

Results of oral administration of quinine sulphate in solution, grains 30, on two consecutive days weekly for 2–12 weeks.

Number of Case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Number of febrile paroxysms (parasitic and non-parasitic) and results of blood examinations during treatment											
			Week of Treatment											
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
564	Apyrexia	—	o	o	o	o	o	o	o	...	...	...	...	...
565	1	2	o	o	o	o	o	o	o	...	...	...	...	...
566	1	1	o	o	o	o	o	o	o	...	...	...	...	...
567	Same day	2–8	o	o	o	o	o	o	o	...	...	...	...	...
568	Same day	1	o	o	o	o	o	o	o	...	...	...	...	...
569	Same day	2	o	o	o	o	o	o	o	...	...	...	...	...
570	Same day	1	o	o	o	o	o	o	o	...	...	...	...	...
571	2	—	o	o	o	o	o	o	o	o	...	...	...	...
572	3	—	o	2°	1°	o	o	o	o	o	...	...	...	...
573	3	1–5	o	o	2*	1	o	1°	o	o	...	...	...	...
574	1	—	o	o	o	o	o	o	o	o	...	...	...	...
575	Apyrexia	—	o	o	o	o	o	o	o	o	...	...	...	...
576	1	—	o	1	o	o	o	o	o	o	...	...	...	...
577	Apyrexia	—	o	o	o	o	o	o	o	o	...	...	...	...
578	Apyrexia	—	o	o	o	o	2	1°	o	o	...	...	...	...
579	Apyrexia	—	o	o	o	o	o	o	o	o	...	...	...	...
580	Apyrexia	—	o	o	o	o	o	o	o	o	...	...	...	...
581	Apyrexia	—	o	o	o	o	o	o	o	o	...	...	...	...
582	1	—	o	o	o	o	o	o	o	o	...	...	...	...
583	Apyrexia	—	o	o	o	o	o	o	o	1°	...	...	...	...
584	1	—	o	o	o	o	o	o	o	o	...	...	...	...
585	1	—	o	o	o	o	o	o	o	o	...	...	...	...
586	Apyrexia	—	o	o	o	o	o	o	o	1°	...	...	...	...
587	1	1	o	o	o	o	o	o	o	o	...	...	...	...
588	1	2	o	o	o	o	o	o	o	o	...	...	...	...
589	1	2	o	o	o	o	o	o	o	o	...	...	...	...

TABLE VII—*continued.*

Results of oral administration of quinine sulphate in solution, grains 30, on two consecutive days weekly for 2–12 weeks.

Number of Case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Number of febrile paroxysms (parasitic and non-parasitic) and results of blood examinations during treatment											
			Week of Treatment											
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
590	Same day	1	0	0	0	0	0	0	0	0	...	...	...	...
591	1	—	0	0	0	0	0	0	0	0	0	...	...	...
592	Apyrexia	—	0	0	0	0	0	0	0	0	0	...	...	...
593	Apyrexia	—	0	0	0	0	0	0	0	0	0	...	...	...
594	Apyrexia	—	0	0	0	0	1°	0	0	0	0	...	...	...
595	1	—	0	0	0	0	1	0	0	0	2	...	...	...
596	Apyrexia	—	0	0	0	3	2	0	0	1°	0	...	...	...
597	3	—	1	0	1°	2	0	2	0	0	0	...	...	...
598	Apyrexia	—	0	0	0	0	0	0	0	0	0	...	...	...
599	Apyrexia	—	0	0	0	1°	0	0	0	0	0	...	...	...
600	Apyrexia	—	0	0	0	0	0	0	0	0	0	...	...	...
601	Apyrexia	—	0	1°	0	0	0	0	0	0	1°	...	...	...
602	Apyrexia	—	0	0	0	0	0	0	0	0	0	...	...	...
603	—	—	0	0	0	0	0	0	0	0	0	...	...	...
604	1	—	0	0	0	1*	0	0	0	0	0	...	...	...
605	1	—	0	0	0	0	0	0	0	0	0	...	...	...
606	1	2–3	0	0	0	0	0	0	0	0	0	...	...	...
607	1	—	0	0	0	0	1*	0	0	0	0	...	...	...
608	2	1	0	0	0	0	0	0	0	1*	0	...	...	...
609	1	1–2	0	0	0	0	0	0	0	0	0	...	...	...
610	1	2–4	0	0	0	0	0	0	0	0	0	...	...	...
611	1	2–5	0	0	0	0	0	0	0	0	0	...	...	...
612	1	1–6	0	0	0	0	0	0	0	0	0	...	...	...
613	1	2	0	0	0	0	0	0	0	0	0	...	...	...
614	Same day	2–7	0	0	0	0	0	0	0	0	0	...	...	...
615	1	3–6	0	0	0	0	0	0	0	0	0	...	...	...

TABLE VII—*continued.*

Results of oral administration of quinine sulphate in solution, grains 30, on two consecutive days weekly for 2–12 weeks.

Number of Case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Number of febrile paroxysms (parasitic and non-parasitic) and results of blood examinations during treatment											
			Week of Treatment											
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
616	1	2	0	0	0	0	0	0	0	0	0	...	...	...
617	1	2	0	0	0	0	0	0	0	0	0	...	...	...
618	Same day	2	0	0	0	0	0	0	0	0	0	...	...	...
619	1	1–2	0	0	0	0	0	0	0	0	0	...	...	...
620	3	1–5	0	0	0	0	0	0	0	1*	0	...	...	...
621	1	1–5	0	0	0	0	0	0	0	0	0	...	...	...
622	Same day	1	0	0	0	0	0	0	0	0	0	...	...	...
623	1	1–3	0	0	0	0	0	0	0	0	0	0	...	...
624	1	—	0	0	1°	0	0	0	0	1°	1°	0	...	...
625	1	—	0	1	2°	3°	0	0	0	0	0	3°	...	...
626	Apyrexia	—	2	0	0	0	0	0	0	1°	0	0	...	...
627	Apyrexia	—	0	0	0	0	0	0	0	0	0	0	...	...
628	1	—	0	0	0	0	0	0	0	0	0	0	...	...
629	Apyrexia	—	0	1*	1*	0	1*	0	0	0	0	1°	...	...
630	1	—	0	0	0	0	0	0	0	0	0	1*	...	...
631	2	—	0	0	0	0	0	0	0	0	0	0	...	...
632	1	1–4	0	0	0	0	1*	0	0	0	0	1	...	...
633	2	2–8	0	0	0	3	0	0	5	6	1	3	...	...
634	2	1	0	0	0	0	0	0	0	0	0	0	...	...
635	2	—	2°	0	P	0	2°	0	0	0	0	0	1°	...
636	Apyrexia	—	0	0	0	0	0	1°	0	0	0	0	0	...
637	1	—	0	0	1	0	1	0	0	1	0	0	2*	...
638	Apyrexia	—	0	0	0	0	0	0	0	0	0	0	0	...
639	1	—	0	0	0	0	0	0	0	0	0	0	0	...
640	2	—	0	0	0	0	1°	0	0	0	0	0	0	...
641	1	—	0	0	0	0	0	0	0	0	0	0	0	...

Vide

TABLE VII—*continued.*

Results of oral administration of quinine sulphate in solution, grains 30, on two consecutive days weekly for 2–12 weeks.

Number of Case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Number of febrile paroxysms (parasitic and non-parasitic) and results of blood examinations during treatment											
			Week of Treatment											
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
642	1	—	1*	1*	2*	2	o	1*	1*	3*	1*	o	1*	...
643	—	—	o	o	o	o	o	1*	o	1*	o	o	o	...
644	1	—	o	o	o	o	o	o	o	o	o	o	o	...
645	2	—	o	P	o	o	o	5	o	o	1*	o	o	...
646	2	1–4	o	o	o	o	o	o	o	o	1*	o	o	...
647	1	2	o	o	o	o	o	o	o	o	o	o	o	...
648	2	2	o	o	o	o	o	o	o	o	o	o	o	...
649	2	—	o	2	2°	1	o	o	o	5°	1°	o	o	o
650	2	—	1*	2°	2°	1*	o	o	o	o	o	o	2°	o
651	1	—	o	o	o	o	o	o	o	o	o	o	o	o
652	Apyrexia	—	o	o	o	o	o	o	o	o	o	o	o	o
653	1	—	o	o	o	o	3	o	o	o	3	o	o	o
654	2	—	o	o	o	o	o	o	o	o	1*	o	o	o
655	2	—	o	o	1	o	o	1*	1	o	o	o	o	o
656	Apyrexia	—	o	o	2*	o	o	o	o	o	o	o	o	o
657	1	—	o	o	o	1*	o	o	o	o	o	o	o	o
658	—	—	...	o	o	1*	o	o	o	1*	1*	o	o	o
659	1	—	o	o	o	o	o	o	o	o	o	o	o	o
660	2	—	o	o	o	o	o	o	o	o	o	o	o	o
661	3	—	o	o	4	o	o	o	o	o	o	o	o	o
662	5	2	3*	o	1°	2°	o	2	1	o	2	o	o	1*

Vide chart

TABLE VIII.

Summary of Table VII.

Grains 30 series.

Week of treatment	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
Number of cases treated	**203**	**203**	**189**	**175**	**156**	**135**	**116**	**92**	**72**	**40**	**28**	**14**
Number of cases having parasitic febrile relapses	6	6	4	11	6	4	3	2	4	2	0	0
Number of cases having non-parasitic febrile attacks	3	3	4	6	3	3	1	5	5	1	2	1
Number of cases having febrile paroxysms not examined	10	16	16	13	7	5	3	6	3	2	2	0
Grand total of all febrile cases ...	**19**	**25**	**24**	**30**	**16**	**12**	**7**	**13**	**12**	**5**	**4**	**1**
Total number of parasitic febrile relapses ...	11	10	8	23	11	11	7	7	8	4	0	0
Total number of non-parasitic febrile attacks	5	3	7	7	3	3	1	7	5	1	3	1
Total number of febrile paroxysms not examined	16	22	24	16	9	6	5	10	3	4	3	0
Grand total of all febrile paroxysms	**32**	**35**	**39**	**46**	**23**	**20**	**13**	**24**	**16**	**9**	**6**	**1**

TABLE IX.

Analysis of TABLE VIII, giving the results for the first 8 weeks.

Grains 30 series.

Week of treatment	1st	2nd	3rd	4th	5th	6th	7th	8th	Average per week
Percentage of parasitic febrile relapse cases per cases treated	3·0	3·0	2·1	6·3	3·8	3 6	2·6	2·2	**3·2***
Number of parasitic febrile relapses per parasitic febrile relapse case	1·8	1·7	2·0	2·1	1·8	2·7	2·3	3·5	**2·2**
Percentage of all febrile (parasitic and non-parasitic) cases per cases treated ...	9·3	12·3	12·6	17·1	10·2	8·8	6·0	14·1	**11·3**
Number of all febrile (parasitic and non-parasitic) paroxysms per febrile case ...	1·7	1·4	1·6	1·5	1·5	1·4	1·9	1·8	**1·6**

* As in many of the febrile relapses no blood examination was made, this figure is undoubtedly too low (*vide* Table VII).

GRAINS 45 SERIES (Cases 663-691)

In these twenty-nine cases the temperature fell to normal on the day of, or 1-3 days after, the commencement of treatment. In eighteen cases parasites disappeared from the cutaneous blood in 1-4 days, and in eleven cases examinations were too infrequent to give a precise figure.

Relapses.

During treatment. Parasitic febrile relapses occurred in two cases (Nos. 668 and 670). The average number of cases which had parasitic febrile relapses each week, over a period of 8 weeks, was ·86 per cent., and that of all febrile cases, parasitic and non-parasitic, was 3·6 per cent. (Table XII). The number of cases on which these figures are based is twenty-nine in the first week and twenty-five in the eighth week.

After treatment. Six of the twenty-one cases observed after cessation of treatment relapsed. Parasites reappeared in 5-18 days, and febrile relapses recurred in 7-18 days, after cessation of treatment. In two of the fifteen cases that did not relapse the observation period was less than 60 days, viz., 10 and 21 days respectively.

TABLE X.

Results of oral administration of quinine sulphate in solution, grains 45, on two consecutive days weekly for 4 to 8 weeks.

Number of Case	Temperature fell to normal in — days after 1st dose	Parasites disappeared from cutaneous blood in — days after 1st dose	Number of febrile paroxysms (parasitic and non-parasitic) and results of blood examinations during treatment — Week of Treatment								Parasitic relapse occurred in — days after cessation of treatment	Febrile relapse (above 100° F.) occurred in — days after cessation of treatment	Observation period in cases which did not relapse
			1st	2nd	3rd	4th	5th	6th	7th	8th			
663	1	2	0	0	0	0	...	...	...	...	Not observed		
664	1	1	0	0	0	0	...	...	...	...			
665	Same day	...	0	0	0	0	...	...	...	...			
666	Same day	...	0	0	0	0	0	...	...	...			
667	1	1	0	0	0	0	0	0	0	0			
668	2	...	0	0	1	0	0	0	0	0			
669	1	2	0	0	0	0	0	0	0	0			
670	Apyrexia	...	0	0	1	0	0	0	0	0			
671	Same day	1–5	0	0	0	0	0	0	0	0	—	—	65 days
672	1	1–2	0	2*	0	0	0	0	0	0	—	—	61 days
673	1	1–6	0	0	0	0	0	0	0	0	5	9	—
674	1	2	0	0	0	0	0	0	0	0	—	—	64 days
675	1	3–9	0	cr.	0	0	0	0	0	0	—	—	60 days
676	2	3	0	0	0	0	0	0	0	0	7–13	—	—
677	1	2	0	0	0	0	0	0	0	0	—	—	65 days
678	1	2	0	0	1*	0	0	P	0	0	—	—	21 days
679	1	1–7	0	0	0	0	0	0	0	0	—	—	65 days
680	2	2–8	0	0	0	0	0	0	0	0	7	7	—
681	3	2	0	0	0	0	0	0	0	0	—	—	65 days
682	1	3–9	0	0	0	0	0	0	0	0	—	—	90 days
683	1	1–7	0	0	1*	0	0	0	1*	1*	14–18	18	—
684	Same day	4	0	0	0	0	0	0	0	0	—	—	71 days
685	1	2	0	0	1*	0	0	0	0	0	—	—	10 days
686	Same day	1–2	0	0	0	0	0	0	0	0	—	—	60 days
687	Same day	2	0	0	0	0	0	0	0	0	7–13	14	—
688	2	2	0	0	0	0	0	0	0	0	—	—	61 days
689	Same day	1	0	0	0	0	0	0	0	0	—	—	60 days
690	Same day	2	0	0	0	0	0	0	0	0	—	—	60 days
691	1	2	0	0	0	0	0	0	0	0	13–15	14	—

TABLE XI.

Summary of Table X.

Grains 45 series.

Week of treatment	1st	2nd	3rd	4th	5th	6th	7th	8th
Number of cases treated ...	**29**	**29**	**29**	**29**	**26**	**25**	**25**	**25**
Number of cases having parasitic febrile relapses	0	0	2	0	0	0	0	0
Number of cases having non-parasitic febrile attacks	0	1	3	0	0	0	1	1
Number of cases having febrile paroxysms not examined	0	0	0	0	0	0	0	0
Grand total of all febrile cases	**0**	**1**	**5**	**0**	**0**	**0**	**1**	**1**
Total number of parasitic febrile relapses	0	0	2	0	0	0	0	0
Total number of non-parasitic febrile attacks	0	2	3	0	0	0	1	1
Total number of febrile paroxysms not examined	0	0	0	0	0	0	0	0
Grand total of all febrile paroxysms	**0**	**2**	**5**	**0**	**0**	**0**	**1**	**1**

TABLE XII.

Analysis of TABLE XI, giving the results for the first 8 weeks.

Grains 45 series.

Week of treatment	1st	2nd	3rd	4th	5th	6th	7th	8th	Average per week
Percentage of parasitic febrile relapse cases per cases treated	0	0	6·9	0	0	0	0	0	**0·86**
Number of parasitic febrile relapses per parasitic febrile relapse case	0	0	1	0	0	0	0	0	**0·12**
Percentage of all febrile (parasitic and non-parasitic) cases per cases treated ...	0	3·4	17·2	0	0	0	4·0	4·0	**3·6***
Number of all febrile (parasitic and non-parasitic) paroxysms per febrile cases ...	0	2·0	1·0	0	0	0	1·0	1·0	**0·62**

* As in this series of cases a blood examination was made on the occasion of every febrile relapse this figure refers to non-parasitic febrile relapses only (*vide* Table X).

COMPARISON OF RESULTS OBTAINED FROM THE VARIOUS TREATMENTS

By means of Table XIII the value of the various treatments as palliatives can be compared.

It will be noted that, as a general rule, the smaller the dose the greater the number of relapses; this is well seen in the Grains 15, 30 and 45 series. The figures given in the Grains 10 series are anomalous, but their value must to a certain extent be discounted owing to the relatively small number of cases observed.

The efficiency of the various treatments, regarded from the palliative point of view, must be judged by the percentage of cases having parasitic febrile relapses whilst undergoing the respective treatments, as we know nothing of the real nature of the non-parasitic febrile attacks. The figure 3·2 in the Grains 30 series has only a minimum value, as in this series the blood was not examined on the occasion of many febrile paroxysms; in the other three series the figures given have an absolute value.

The figures given in line 3 referring to the percentages of all cases having febrile paroxysms (parasitic and non-parasitic) are strictly comparable, and therefore are of considerable value.

TABLE XIII.

Comparison of palliative results obtained from the different treatments.

	Grains 10	Grains 15	Grains 30	Grains 45
Percentage of parasitic febrile relapse cases per cases treated (average per week)	6·3	18·8	3·2*	0·86
Number of parasitic febrile relapses per parasitic febrile relapse case (average per week) ...	1·6	2·0	2·2	0·12
Percentage of all febrile (parasitic and non-parasitic) cases per cases treated (average per week) ...	10·4	20·1	11·3	3·6
Number of all febrile (parasitic and non-parasitic) paroxysms per febrile case (average per week)	1·5	1·9	1·6	0·62

* The figure (3·2) representing the weekly average percentage of cases treated which had parasitic febrile relapses is undoubtedly too small, as in this series the blood was not examined in many of the febrile paroxysms.

As stated in previous studies, it was our aim to observe all cases treated for a period of at least 60 days after cessation of treatment. This is, of course, a purely arbitrary period determined by conditions over which we had no control. Had the cases always been observed for this period it would be possible to compare the results of the various treatments employed, the information obtained from any of the treatments reading as follows:—Number of cases treated x, number of cases which relapsed in an observation period of 60 days y, percentage of cases which relapsed z.

It is obvious that if certain cases were observed for only 20 days after cessation of treatment whilst others were observed for 60 days, the results cannot be compared. Consequently, in considering the percentage of relapses observed after the various forms of prolonged (*interrupted*) treatment adopted by us it is necessary, as unfortunately some of the cases were not observed for the full period of 60 days after treatment to give two sets of figures:—(1) Minimum figure, i.e. the percentage actually observed to have relapsed; (2) Maximum figure, i.e. one based on the assumption that all cases not observed for a period of 60 days relapsed before the expiration of that period. It is clear that the true percentage of cases which would relapse in an observation period of 60 days must lie somewhere between these two extremes, probably, for reasons which we shall consider in a future paper, much nearer the minimum than the maximum value.

The superiority of the Grains 45 treatment is clear, as the maximum percentage of relapses possible in an observation period of 60 days is less than the observed minimum values in the Grains 10 series (Table XIV).

The curative value of Grains 45 on two consecutive days weekly for a period of 8 weeks—no relapse in an observation period of 60 days being the criterion of cure—lies between 71·4 and 61·9 per cent.

TABLE XIV.

Comparison of curative results obtained from the different treatments.

Dose in grains	Duration of treatment	Number of cases treated	Number of cases which relapsed	Number of cases not relapsing, but observed for less than 60 days	Percentage of cases which relapsed	
					minimum	maximum
10	8–16 weeks	17	7	10	41·2	100·0
45	8 weeks	21	6	2	28·6	38·1

TOLERANCE OF THE TREATMENT

All the patients were able to take without difficulty the largest dose of quinine employed, namely Grains 45 (three doses of Grains 15) on two consecutive days weekly.

CONCLUSION

Of the various forms of *interrupted* treatments used by us, that of Grains 45 is the best.

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STUDIES IN THE TREATMENT OF MALARIA

IX. A COMPARISON OF THE RESULTS OF INTERRUPTED AND CONTINUOUS QUININE ADMINISTRATION

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In two previous papers we have recorded the results (palliative and curative) obtained by *continuous* and *interrupted* quinine administration respectively, i.e. on the one hand by a daily dose of quinine and on the other by two doses weekly, over a given period.

We may recapitulate here our previous conclusions.

In Paper VII (1918) the conclusion reached was that 'of the various forms of *continuous* treatments used by us that of Grains 45 is the best.'

In Paper VIII (1918) the conclusion reached was that 'of the various forms of *interrupted* treatments used by us, that of Grains 45 is the best.'

In the present paper we propose to compare these results so as to form an estimate of the respective values of the two modes of treatment.

We shall compare the results obtained: (1) During treatment (palliative). (2) After treatment (curative).

I. DURING TREATMENT

We have at our disposal for this purpose the following data:—

1. Results of administration of grains 30 daily for eight weeks.
2. Results of administration of grains 45 daily for eight weeks.
3. Results of administration of grains 30 on two consecutive days weekly for eight weeks.
4. Results of administration of grains 45 on two consecutive days weekly for eight weeks.

In Table I, for purposes of convenience and comparison, the data are given together.

TABLE I.

Comparison of palliative results obtained by *continuous* and *interrupted* treatments.

	Grains 30		Grains 45	
	Continuous	Interrupted	Continuous	Interrupted
Percentage of parasitic febrile relapse cases per cases treated (average per week over 8 weeks)	7·2	3·2*	1·8	0·86
Number of parasitic febrile relapses per parasitic febrile relapse case (average per week over 8 weeks)	0·7	2·2	0·1	0·12
Percentage of all febrile (parasitic and non-parasitic) cases per cases treated (average per week over 8 weeks) ...	17·3	11·3	10·0	3·6
Number of all febrile (parasitic and non-parasitic) paroxysms per febrile case (average per week over 8 weeks) ...	1·0	1·6	1·0	0·62
Amount of quinine sulphate in grains per week	210	60	315	90
Amount of quinine sulphate in grains per 8 weeks	1680	480	2520	720

* The figure (3·2) representing the weekly average percentage of cases treated which had parasitic febrile relapses is undoubtedly too small, as in this series the blood was not examined in many of the febrile paroxysms (*vide* Paper VIII).

In both Grains 30 and Grains 45 series the palliative results of the *interrupted* treatment are better than those of the corresponding *continuous* treatment.

Comparing all febrile paroxysms the *interrupted* treatment likewise gave better results.

It should be noted (1) that in the Grains 30 series (*continuous* treatment) the number of cases on which the figures are based was fourteen in the first week and eight in the eighth week; (2) that in the Grains 30 series (*interrupted* treatment) the number of cases was two hundred and three in the first week and ninety-two in the eighth week; (3) that in the Grains 45 series (*continuous* treatment) the number of cases was nineteen in the first week and seven in the eighth week; and (4) that in the Grains 45 series (*interrupted* treatment) the number of cases was twenty-nine in the first week and twenty-five in the eighth week.

Although, then, the number of cases in Series 1, 3 and 4 is small, yet in both forms of treatment the superiority of Grains 45 is clearly indicated, and as in both Grains 45 and Grains 30 series the figures for the *interrupted* treatment are better than the corresponding figures for the *continuous* treatment, we conclude that the *interrupted* method is not of less value than the *continuous* method.

II. AFTER TREATMENT

We have at our disposal for this purpose the following data:—

1. Results of administration of grains 30 daily for five to eighteen weeks (43 cases).

2. Results of administration of grains 45 daily for sixteen days to eight weeks (19 cases).

3. Results of administration of grains 45 twice weekly for eight weeks (21 cases).

The figures are given in Table II. It must be distinctly understood that all the figures in this table are based on an observation period after cessation of treatment of sixty days.

TABLE II.

Comparison of curative results obtained by *continuous* and *interrupted* treatments.

	Grains 30				Grains 45			
	Continuous		Interrupted		Continuous		Interrupted	
Number of cases treated	43		—		19		21	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Number of cases which relapsed* ...	34	39	—	—	7	10	6	8
Percentage of cases which relapsed ...	79·1	90·7	—	—	36·8	52·6	28·6	38·1

* As explained in Papers VII and VIII, the minimum figure refers to the relapses actually observed, whilst the maximum is the sum of these and of such cases as left hospital before the completion of the 60 days observation period.

In the Grains 30 series (*interrupted* treatment) the results after treatment were not observed, but it is noteworthy that the number of relapses after *continuous* treatment with grains 30 daily for two months lies between 79·1 and 90·7 per cent.

In the Grains 45 series the *interrupted* treatment gives a smaller percentage of relapses than the *continuous* treatment, possibly because in the *continuous* set many of the cases could not (owing to intolerance) complete the full course of eight weeks treatment, or possibly the difference may be of no significance owing to the small number of cases observed.

However, we can with reason compare the results obtained by the *interrupted* Grains 45 series with those obtained by the *continuous* Grains 30 series. The patients of the former group received grains 90 of quinine weekly, whereas those of the latter group had grains 210 weekly. In spite of the fact that the Grains 30 series (*continuous* treatment) received two and a third times as much quinine weekly as the Grains 45 series (*interrupted* treatment), the results given by the latter treatment are strikingly better, only 28·6 to 38·1 per cent. relapsing as against 79·1 to 90·7 per cent. in the case of the Grains 30 series (*continuous* treatment). This superiority is as striking when the two treatments are compared from the palliative point of view, as previously shewn.

Finally we may compare the two modes of treatments from two other points of view:—

III. TOLERANCE

A daily dose of grains 45 is not well borne by all patients, and in fact in twelve of nineteen cases the treatment had to be abandoned on account of vomiting and tremors before completion of the eight weeks, but in no case did the administration of grains 45 twice weekly produce any intolerance, all the cases who remained in hospital completing the eight weeks treatment without difficulty.

IV. ECONOMY OF QUININE

The *interrupted* treatment requires over any given period only two-sevenths of the quantity of quinine required for the corresponding *continuous* treatment.

CONCLUSIONS

Interrupted treatment with quinine grains 30 or 45 twice weekly is preferable to *continuous* treatment with quinine grains 30 or 45 seven times weekly. Grains 45 twice weekly is better than grains 30 twice weekly or than grains 30 daily, both as a palliative and as a curative treatment.

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A COMPARATIVE STUDY OF THE HABITS OF *GLOSSINA BREVIPALPIS*, NEWST., *G. FUSCA*, WEST., AND *G. PALLIDIPES*, AUST. IN THE BELGIAN CONGO

BY

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(WITH 1 MAP AND 1 CHART)

(*Received for publication Nov.* 16, 1917)

INTRODUCTION

Comparatively little is known about the habits of *Glossina fusca* and *G. pallidipes*, although those of *G. brevipalpis* have been more fully investigated. Austen, in his monograph, gives a résumé of the literature on tsetse-flies, but, apart from a few records of captures, I have found nothing relating to the habits of either *G. fusca* or *G. pallidipes*. This lack of knowledge may be explained by the apparent rarity of these species, as at present the captures recorded are comparatively few. With *G. fusca* this is particularly so, and all observers, myself included, have agreed that usually only one or two specimens at a time are to be found in a given spot. However, this is incorrect, as in regions where *G. fusca* occurs it is frequently not at all uncommon, and several dozens may be collected in a place at any time of the day. Still, it does not swarm in such large numbers as *G. brevipalpis*, and only a close acquaintance with its peculiar habits enables one to discover it in any quantity. *G. pallidipes*, also, may be found in large numbers, sometimes almost as great as those of *G. morsitans*, if a knowledge of the habits characteristic of the species be used for its detection. By following directions based on such a knowledge my two trained boys have collected in a few weeks over five hundred examples of *G. fusca* from the surroundings of Katompe alone. Many

points concerning the habits of *G. brevipalpis* also require further investigation, and of these one of the most important relates to its distribution. It has often been stated that this tsetse-fly is much restricted in its distribution, and that although found chiefly near villages and watercourses it is not entirely confined to such situations. The accuracy of this statement is questionable, and in an article on the habits of this species I have given reasons why it is only found near villages, even though it may also occur elsewhere. Recent observations have confirmed my conjectures, for I have found that *G. brevipalpis* is by no means confined to particular localities, but that it occurs over large tracts of country. Except in clearings, for example, it exists everywhere between Katompe and Kabalo—a distance of 65 kilometres—and between Katompe and Kongolo (about 125 kilometres).

Although clearness and brevity would perhaps benefit by discussing separately the three species of tsetse-flies treated in this paper, this has not been done for two reasons. First, my observations on these species have been made simultaneously, and by describing the more important and conclusive in detail, instead of giving a brief résumé, points requiring further elucidation will be rendered more conspicuous. Second, a comparative description will give prominence and make easily distinguishable those habits which are common to all three species, and those which are peculiar to each.

My observations were made, for the most part, at the Agricultural Station of Katompe, where there are coffee plantations. This Station (in the province of Katanga, Tanganika-Moere district) is situated almost midway between the River Lualaba and the River Lomami, slightly south of the 6th S. parallel and about 26° 50′ E. longitude. Katompe was selected as a suitable spot for this research because, when paying a brief visit to the station in June 1914, five species of *Glossina* were found. On this occasion numerous examples of *G. brevipalpis* were seen, and my native boys captured a number of *G. pallidipes*, several *G. fusca*, and a few *G. morsitans* and *G. palpalis*. Three months (February to April, 1916) were devoted to the work, and the observations made were controlled and completed by expeditions taken to the same district:—(1) From Katompe to Kabalo and return; (2) Katompe to Kongolo; and (3) Kongolo to Kabinda. All the observations

were made during the rainy season, and, unfortunately, their hoped for completion and extension by means of investigation throughout the dry season was prevented, for the time being, by pressure of other work.

In previous articles on *G. morsitans* and *G. palpalis*, I have emphasised the importance of the various types of vegetation in determining the distribution of tsetse-flies in general and of one or other species in particular. The terminology used in connection with the different types of tropical vegetation is somewhat confusing, and in these papers I have been constrained to give my own interpretation of the terms used. Here, it will be sufficient to state that the main types of Tropical African vegetation may be grouped in three classes:—(1) Forest (Forêt); (2) Woods or Park-land (Bois ou parc); and (3) Savannah (Savane); and that the last-named class may be sub-divided into Grassy Savannah (Savane herbeuse) and Wooded Savannah (Savane boisée). These types are, of course, not sharply or abruptly separated from one another, and areas exist in which the vegetation is intermediate or transitional in type, so that qualification of the above terms (e.g. forêt dense, forêt peu dense, bon parc, petit parc, parc dégénéré, savane bien boisée, savane peu boisée, etc.) is sometimes necessary in describing a particular spot.

I have previously pointed out that although all tsetses require shade, and are therefore never found in the stretches of grassy savannah, different species require different kinds of vegetation. Thus, *G. morsitans* only occurs in park-land, while *G. palpalis* is only found near water bordered with forest (galérie forestière).*

The investigations herein recorded were undertaken, therefore, in order to discover, *inter alia*, the types of vegetation necessary for *G. brevipalpis*, *G. fusca*, and *G. pallidipes*; whether these flies are capable of adapting themselves to any kind of arborescent vegetation—be it forest, park or wooded savannah—or whether all or any of them require one of these three types. A further object, also, was to determine the importance of water as a factor influencing their distribution.

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* As *G. palpalis* does not form the subject of this paper, I would note that this reference applies particularly to the region of the upper Lomami R.; in other regions I have found this species near water where there is no true forest, and notably in places where tall reeds and shrubs form a complete arch over the water.

KATOMPE AND NEIGHBOURHOOD (see chart)

The Agricultural Station of Katompe is situated at an altitude of from 800 to 850 metres, and consists of a few houses surrounded with fruit trees and coffee plantations. The station and plantations form an artificial clearing and are surrounded by a belt of forest 1,000 metres wide. North of the station, between the inner edge of the forest and the plantations is a belt of rubber trees ('Ireh'—*Funtumia elastica*) some six years old; southwards, and similarly situated, are rather numerous rubber-yielding lianes (*Landolphia*). About 500 metres to the north-east the marshy stream Namvula (an indirect tributary of the River Lualaba) flows through the forest, and at a similar distance to the north and north-west, in the same forest belt, is a small swampy affluent of the Namvula—the Kingoï. Beyond and encompassing the forest belt is a zone—approximately 1,000 metres wide—of open country, containing but few trees (savane très peu boisée). Beyond this again the vegetation is of a variable nature, as follows:—westwards, an area, about 3,000 metres wide, of transitory vegetation (savane boisée—petit parc) extending to a small tract of forest bordering the stream Luangoï; northwards the open country is continued as far as the Namvula forest-belt, a distance of some four kilometres; to the south-east (towards Kabalo) and north-east (towards Kongolo) the vegetation is again transitional in type, and consists of small areas of park and wooded savannah, alternating with clearings, the whole about 3,000 metres wide and reaching to a tract of forest some 500 metres broad. Park-land commences a short distance beyond this forest belt and stretches as far as the River Lualaba.

It should be noted that the forest region last referred to is evidently an extension of that of the River Namvula, although widely separated therefrom; also that those portions of the forest belt crossed by the routes to Kongolo and Kabalo are situated one to two kilometres from any water. This circumstance is of considerable importance from the point of view of this study.

In order to overcome some of the difficulties attendant upon the observation and capture of tsetse-flies, my carriers (who had been with me for a considerable period and were well acquainted with these insects) had standing orders to look for these flies, and their endeavours in this direction were stimulated by the payment of 1 to 2 centimes per fly. This arrangement enabled me not only to obtain very large collections of tsetse-flies, but greatly reduced the chances of overlooking their presence. I had with me also two native boys who had been continually employed in this work for three years and who consequently were fully trained. It is not too much to say that without their help these results could not have been obtained, and that, thanks to their keen sight and their bare legs and arms (factors of much importance in tsetse-fly investigations), my instructions were executed with success.

First Observation

Between 5 p.m. and sunset my boys captured several hundred specimens of *G. brevipalpis* hovering over the ground on the paths of the forest belt surrounding Katompe. This species was swarming in such places before sunset. A single specimen of *G. fusca* was found among the *G. brevipalpis*. No flies were taken in these spots during the early morning either by the boys or myself, in spite of careful searching extended over several days. Other evening expeditions supplied numbers of *G. brevipalpis* and a few *G. fusca*. The specimens of *G. brevipalpis* collected were all males, but both sexes of *G. fusca* were represented among those caught.

Second Observation

During the evening numerous *G. brevipalpis* and a certain number of *G. pallidipes* (♂s and ♀s) were collected near the ground in the region beyond the forest. When returning and crossing the Namvula forest belt, two specimens of *G. fusca* were captured attacking natives. No tsetses were found in these spots the following morning or afternoon. *G. brevipalpis*, therefore, exists outside the forest belt and at a considerable distance from water and habitations. *G. pallidipes* occurs in the park-land and even in the wooded savannah, but not in the forest proper; it seems to have similar habits

to *G. brevipalpis* in that it is active in the evening and flies near the ground. *G. fusca* inhabits the forest near water, as does *G. palpalis*; its activities are apparently confined to the evening, but less markedly than those of *G. brevipalpis*. It seems also more voracious than the latter species.

Third Observation

Two specimens of *G. fusca* were taken in the morning, one at 7 a.m., the other at 11 a.m. The first had settled on my clothes when I was in the vicinity of a brook on the road towards the River Kingoï. Two days later another example was captured on a shady portion of a wall behind my house, and lastly on two occasions a specimen was taken on a native's leg (on the Kingoï road) at 7 a.m. Thus *G. fusca* seems to be active in the morning and even throughout the day, eventually leaving the immediate vicinity of water and forest.

Fourth Observation

In order to demonstrate the non-confinement of *G. brevipalpis* to the immediate neighbourhood of villages, I decided to observe the fly closely along the Kabalo route as far as a spot five kilometres distant from the station where there is a wide belt of forest. The journey was begun at 2.45 p.m., and no flies were seen until we had passed through the forest near the station and the savannah beyond. Then at 3.15 p.m., at the commencement of thin park country (petit parc), a *G. pallidipes* was seen hovering over the ground. From now onward the number of flies increased until at 3.45 p.m. they were very numerous, flying near the ground in the same way as *G. brevipalpis*. Their flight and the accompanying humming, however, was more feeble than that of *G. brevipalpis*, and consequently they were not so easily observed. However, the flies were only seen in shady places, and never in clearings where they would be exposed to the full force of the sun. None attempted to bite the natives, but one settled for an instant on my shoulder. At 4.30 p.m. a *G. brevipalpis* was seen, and towards 5 p.m. this species was abundant. At 5.15 p.m. *G. brevipalpis* was much more numerous than *G. pallidipes*, and the latter species had completely disappeared by 5.30 p.m. (half an hour before sunset). *G. brevi-*

palpis continued active till shortly after sunset along the whole route from the station to the forest belt (a distance of five kilometres), except, of course, in the open country. *G. brevipalpis*, therefore, occurs at a considerable distance—several kilometres at least—from all habitations and water. It appears that *G. pallidipes* has similar habits to *G. brevipalpis*, but commences and ceases its activities earlier than the latter. *G. brevipalpis* seems to occur equally in forest, park, and even wooded savannah, while *G. pallidipes* apparently inhabits the park and wooded savannah, but not the forest.

Fifth Observation

In order to ascertain whether *G. pallidipes* is active throughout the day, the known haunts of the fly were visited on several occasions, the first day from 6 a.m. to 10 a.m., the second day from 10 a.m. to 2 p.m. and the third day from 2 p.m. to 6 p.m. This procedure was carried out first of all on the Kabalo route, and later on others. In general no sign of *G. pallidipes* was seen until 3 p.m., when it appeared in small numbers hovering over the ground; its numbers gradually increased, until the maximum abundance was reached from 4 to 4.30 p.m., slightly before *G. brevipalpis* made its appearance. Shortly before or after 5 p.m. (one hour before sunset), according to the density of the foliage, *G. pallidipes* disappeared. Such is the general rule regarding the activity of this species, but occasionally specimens may be seen in flight earlier in the day—from 11 a.m. onwards.

Sixth Observation

Among the many hundred *G. brevipalpis* captured during the evenings it was exceptional to find more than one or two females, although of the *G. pallidipes* captured under the same conditions from 15 to 20 per cent. were females.

Seventh Observation

A thorough search was made for the resting-place of *G. brevipalpis* during the daytime. The herbage and vegetation generally were searched, but particular care was given to the examination of the trees, since, as a rule, it is only where these occur

that tsetse-flies are found and I had noticed on many occasions that at the time of disappearance of *G. brevipalpis* numbers appeared to fly towards the trees. After many failures I decided to give up searching the herbage and direct my attention to the trees. Thinking that perhaps the leaves would provide the best hiding places, careful examination of them was made and the branches shaken. Both upper and lower parts of the trees were examined. These investigations were made in a spot situated in the park on the Kabalo route where *G. brevipalpis* was exceptionally numerous during the evenings. After two days of laborious and unfruitful search two of these flies were seen resting on tree trunks about two metres above the ground. Each fly (one a ♂, and one a ♀) was resting in a vertical position with its head pointing downwards.

Eighth Observation

Searching the same place from 9 a.m. to noon the next day, a score of the flies were discovered, but only ten captured, two of which were females. Before giving details relating to *G. brevipalpis* in repose certain remarks are necessary. The trees forming the park are almost always more or less stunted and twisted, the trunk is sometimes very oblique and often branches one or two metres from the ground, so that no large central trunk is present. Now and then a smaller and thinner trunk may be seen growing by the side of the principal trunk and arising (below or just above ground) from the same root. Though uncommon in park-land, large lianes occur intertwined among the branches and tree trunks. The bark of the trunks, larger branches, and lianes, is thick and extremely rugose, the colour (as a result of bush fires) is greyish-yellow and sometimes blackish; these dull colours of the tree-trunks harmonize with those of the fly, and, owing to the irregularities of the bark, the resting tsetses are rendered almost invisible. In such surroundings *G. brevipalpis* selects places on the trunks one to three (usually two) metres above the ground, so that generally it is necessary to examine portions of the tree above one's own level. The flies always rest in the shade, and are usually found on trees bearing considerable foliage. The trunks, larger branches and larger lianes are usually selected for resting purposes by the flies

which, unless the branch be perfectly horizontal, *always place themselves so that their heads are directed downwards.* If the branch be horizontal or slightly oblique the flies generally choose the lower surfaces for resting, and thus obtain a greater amount of shade. They are never found on or under leaves or on small branches, and although when active keep near the ground, scarcely ever rest low down on the trees. *G. brevipalpis* remained perfectly still when resting, and although carefully watched on one occasion for more than an hour, made no movement during that time. A fly may even be closely approached and one's hand placed within a few centimetres from it without alarming it in the least. In spite of this they are not easily captured, and when disturbed fly quickly away for a short distance to settle again, as a rule, on another part of the same tree or branch. Frequently three or four flies are found resting on the same tree or branch, sometimes one beside the other.

Ninth Observation

The above observation was repeated several times in different places, and at all hours of the day from 6 a.m. with same result. The sexes of the flies thus captured were as follows:—

			♂	♀
11	*G. brevipalpis*	=	7	4
2	,, ,,	=	1	1
2	,, ,,	=	2	0
5	,, ,,	=	5	0
2	,, ,,	=	1	1
10	,, ,,	=	8	2

Later at the same place eighty-nine specimens were captured on the wing, and all were males. Although, therefore, females may be captured by searching the resting places, they are nevertheless comparatively rare.

Tenth Observation

In all those haunts of *G. brevipalpis* where search was made for resting places, *G. pallidipes* was seen on the wing during its time of activity. Examination of the trees showed that it selected the same kind of situations for resting purposes as *G. brevipalpis*, and further that the two species settled on the same tree and even side by side. The resting position of *G. pallidipes*, however, differs somewhat from

that of *G. brevipalpis* in that *G. pallidipes* very frequently rests with the head directed upwards. Examples showing the distribution of sexes among resting *G. pallidipes* are:—

		♂	♀
13	*G. pallidipes* =	11	2
3	„ „ =	1	2
12	„ „ =	8	4

Eleventh Observation

Examination for resting places of *G. fusca* was made in the forest belt surrounding Katompe near the River Namvula, where the fly was known to occur. The results were surprising in view of the apparent rarity of this fly; fifteen *G. fusca* (8 ♂s, 7 ♀s) and two examples of *G. brevipalpis* being collected in two hours along a stretch of the path not exceeding 100 metres.

It may be noted here that the bark of the forest trees differs considerably from that of the trees in the park and wooded savannah. The bush fires do not reach the forest, and thus the tree trunks preserve their natural colour (greyish-green), and the bark remains smooth. Tsetse-flies resting on the forest trees are much more easily seen than those resting on trees in other regions. Moreover, *G. fusca* is more easily visible than *G. brevipalpis* by reason of its darker colour, which renders it more conspicuous against the bark. These advantages, however, are to a certain extent counterbalanced by the poorer light and by the difficulties of advancing through the thick vegetation. *G. fusca* rests in exactly the same manner as *G. brevipalpis*, i.e. whenever possible, with the head pointing downwards; like *G. brevipalpis* and *G. pallidipes* it is found at a height of from one to two and a half metres (and probably higher), but sometimes occurs only half to one metre above the ground. Also *G. fusca* very often rests on the smaller lianes, even more frequently perhaps than on tree trunks. No attempt to bite was made during the two hours (7 to 9 a.m.) we were engaged on this investigation.

Twelfth Observation

The above observation was repeated on several days, at different times and in different places in the forest belt with the same result, except that on three occasions the flies attacked us. The proportion

of the sexes among the specimens of *G. fusca* captured when resting were:—

				♂	♀
7	*G.*	*fusca*	=	1	6
32	„	„	=	23	9
15	„	„	=	8	7
11	„	„	=	6	5
7	„	„	=	2	5
17	„	„	=	10	7
15	„	„	=	12	3
15	„	„	=	7	8
12	„	„	=	9	3

The proportion of females therefore is variable, but always high. *G. fusca* does not appear to swarm in such large numbers as *G. brevipalpis* but occurs in forest belts near water in moderately large numbers, and several dozen examples may be captured in a short time at a particular spot. The fact that but few are captured on the wing tends to show that in general this species is not very active, or rather that it rarely attacks passers-by. It remains to be seen whether *G. fusca* is really somewhat inactive or whether it also has its definite hour of activity, but the evidence seems against this, as in spite of being present in the known haunts of *G. fusca* on various occasions at all times from sunrise to sunset, very few attacks were made by these flies.

Thirteenth Observation

An accident put me on the track of the true habits of *G. fusca*. The belt of rubber trees, mentioned on p. 368, situated at the edge of the forest and north of the station, is quite near my house. These trees are fairly tall and provide considerable shade, and the area they occupy (some 600 to 700 metres from the River Namvula) being completely cleared some six years ago, nothing but rubber trees are now visible. On walking through this spot one day at 9 a.m., two tsetse-flies were observed on a tree-trunk; one, a *G. fusca*, was resting half a metre from the ground, and the other, a *G. brevipalpis*, one metre higher. Later, several other flies of both species were found on trees in this place. From this it would seem that *G. fusca* is not attracted by any particular type of vegetation as long as dense shade is provided, and therefore plantations containing trees with dense foliage may take the place of forest, and those containing

trees with less thick foliage may replace park-land in the economy of tsetse-flies.

Fourteenth Observation

From the foregoing observation it seems that *G. fusca* occurs at a considerable distance from water: but whether dense shade such as obtains in the forest is alone sufficient for its existence is questionable. The forest surrounding the station is traversed by one or two streams, and the tracts of forest five kilometres to the north and west are also crossed by streams; but those which are found at equal distances from Katompe, on the Kabalo and Kongolo routes are about two kilometres from the River Namvula. Both these are typical forest belts about 500 metres wide, and an attempt was made to determine whether the flies found in these belts were merely stray specimens or not. The forest belt on the Kongolo route was visited from 7 to 10 a.m., and ten specimens of *G. fusca* (♂s and ♀s) were captured resting on trees or lianes—none were seen on the wing. Between this tract of forest and that surrounding Katompe, in the patches of park and wooded savannah, we took several resting examples of *G. brevipalpis* and *G. pallidipes*, but not a single *G. fusca*. The latter species therefore, like *G. palpalis*, requires dense foliage, but apparently is not so dependent upon water. *G. fusca*, however, only inhabits fairly wide tracts of forest, while *G. palpalis* contents itself, if necessary, with but few trees. In this connection it must be stated that between the River Lualaba and the upper Lomami River—the region to which the reference to *G. palpalis* holds—forest belts without rivers or streams are very rare.

Fifteenth Observation

The above observation was repeated on the two days following. The first visit was made in the morning, but up to 2 p.m. only two specimens were found at rest on the trees and lianes near the road. The second visit was made between 4 and 6 p.m., during which time twenty specimens were collected. *G. fusca*, therefore, was found more easily during the morning and evening than during midday between 11 a.m. and 2 to 3 p.m. In order to discover the reason for this we paid another visit at noon to the selected spot,

but careful search along the route revealed nothing. Examinations were then made of the thickets situated a few metres from the path, and in a short time numerous flies (about twenty) were seen. Owing, however, to the profuse vegetation, capture was made difficult and only two examples were obtained. The sole difference between these two situations is that along the path itself the lianes are less numerous and do not entirely prevent the penetration of the sun's rays, but, a short distance from the path, the vegetation is much more dense, so that even at mid-day these retreats are always cool and shady. *G. fusca*, then, is similar to *G. brevipalpis* in that it often changes its resting place during the day in order to seek a cool and more shady position and escape the heat of the sun.

Sixteenth Observation

While making the above investigations on *G. fusca* we were sometimes attacked by one, two, or even three of these flies, which afterwards followed us for a considerable distance. This happened specially during the morning and evening, but sometimes during mid-day. On such occasions *G. fusca* flew like *G. palpalis* and *G. morsitans*, and did not hover near the ground like *G. brevipalpis* or *G. pallidipes*. These differences of flight were rendered more noticeable between 5 p.m. and 6 p.m. when both *G. fusca* and *G. brevipalpis* were active; the latter were very numerous, flew low, and paid no attention to us at all, while the few *G. fusca* flew round us and tried to bite. Other examples of *G. fusca* continued to rest throughout this time on the trees and lianes. Moreover, *G. fusca*, although larger, flies more silently than does *G. brevipalpis*.

Seventeenth Observation

The above observation is confirmed by numerous facts. The natives of Katompe bring me occasional specimens of *G. fusca* captured at all times throughout the day; they are always taken on the wing evidently intending to bite, and are never found hovering near the ground. On one occasion, when a European arrived at the station with a number of carriers, I was given several specimens of *G. fusca* which had been caught within the station itself, and which had evidently followed the travellers. A specimen of *G. pallidipes* was also found in the station during the evening of the same

day. *G. fusca* seems to be more voracious and to travel farther than *G. brevipalpis;* its repose during the day is much more easily disturbed than that of the latter species, and perhaps than that of *G. pallidipes* also.

Eighteenth Observation

Although rarely, *G. pallidipes* has been captured with *G. brevipalpis* on the paths of the forest surrounding Katompe, and on the Kabalo route. *G. pallidipes* occurs normally almost everywhere, except in the forest belts, and such occasional specimens as have been found in these places, are evidently individuals that have wandered.

Nineteenth Observation

Several specimens of *G. fusca,* captured on the wing during the morning when in search of blood, were brought to me by some native workmen; these workmen (thirty in number) were clearing part of the forest adjacent to the coffee plantations. They had commenced by cutting the undergrowth, lianes, creeping plants, bushes, etc., and for the time being had left the larger trees. On paying a visit to this spot at 7 a.m., numerous examples of *G. fusca* (twenty at least) were found resting on the trees. As previously observed, they were resting, often side by side, from one to two and a half metres above the soil, with their heads directed downwards. At 8.30 a.m., the sun's rays commenced to penetrate between the branches, and the tsetses flew away. The majority evidently sought other trees, but a few tried to attack us. Those that flew off went a considerable distance, and did not alight again near by as *G. brevipalpis* or *G. pallidipes* would have done. Of those which flew round us, fifteen (9 ♂s, 6 ♀s) were caught, two of which were greatly distended with blood. About 9 a.m. *G. fusca* disappeared, and until noon we did not see another specimen either active or resting. Three weeks later another visit was paid to this spot; the undergrowth had all been cut in the meantime, and no labourers had been near for ten days. Between 6.30 and 9 a.m., three specimens of *G. fusca* and three of *G. brevipalpis* were captured on the trees, but none were seen on the wing. At the same time on the following day, however, four *G. fusca* attacked us and were captured; the morning was cool

and misty owing to a heavy rain which had fallen the previous evening. On the whole, however, *G. fusca* was much less abundant in this spot than it was a few weeks earlier when the flies were undoubtedly attracted by the presence of the labourers.

Twentieth Observation

In view of the fact that recent observations showed that *G. brevipalpis* and *G. pallidipes* were widely distributed around Katompe, an expedition to Kabalo was undertaken in order to discover whether a similar distribution of the fly was present on this route. Incidentally, observations on the limits of *G. morsitans* in this region were desired, as opportunities for the investigation of this question to the east of Katompe had not occurred previously. The distance from Katompe to Kabalo is about 65 kilometres, and in order to carry out a thorough examination of the route, the journey was made in short stages and no stop made in the same village on both outward and return journeys. Apart from several clearings of greater or less extent, the whole route is well wooded, although neither uniformly nor typically so. The greater part of the route lies through park-land, but the larger or smaller stretches of typical park, often alternate with stretches of thin park-land (parc dégénéré). No forest, except that five kilometres from Katompe, occurs along the whole route; even near the rivers the vegetation is but slightly denser and still park-like in nature. From Katompe to Kabalo, on the River Lualaba, no *G. palpalis* were seen, and not more than one *G. fusca* since leaving the Katompe forest-belt. On the other hand, *G. brevipalpis* and *G. pallidipes* occurred *everywhere* along the whole route (except, of course, where there were no trees) but varied in abundance according to density of shade. A short distance from the forest belt previously mentioned, notably from the village Kayumba (see map), *G. morsitans* made its appearance, and thereafter occurred along the whole route as far as the River Lualaba. Except in those places where the park was of a typical nature, *G. morsitans* was comparatively scarce, hardly troubled one, and frequently could only be discovered by searching. *G. pallidipes* occurred in about the same numbers as *G. morsitans*, but *G. brevipalpis* was widely distributed and present in greater numbers than the other two species together. The habits of these three

species—those which are common to all three, and those which are peculiar to one—may be given here. During the early morning no flies were seen on the wing. and if the morning were cool and fresh as after a heavy rain, the absence of the flies lasted till 8 or 9 a.m. However, numerous specimens of all three species were constantly observed resting on the tree trunks and larger branches, often all three on one tree, but *G. brevipalpis* was particularly in evidence, since it was more abundant in this region than the other species, and its larger size rendered it more easily visible. *G. brevipalpis*, as before rested with its head pointing downwards, *G. morsitans* with its head upwards, and *G. pallidipes* with its head sometimes (more often) upwards, sometimes downwards.

Among the *G. brevipalpis* captured on trees, females were often found, but never formed more than 10 per cent. Between 7 and 9 a.m., according to the temperature, *G. morsitans* became active, and remained so, if no rain fell, during the rest of the day. This was not the case, however, with either *G. brevipalpis* or *G. pallidipes*. During eight days' travelling (from 6 to 10 or 11 a.m. each day) along this route no *G. brevipalpis* were ever seen on the wing, and on two occasions only were specimens of *G. pallidipes* noticed, both of which had settled on me. In the neighbourhood of villages where halts were made, the presence of *G. pallidipes* and *G. brevipalpis* would naturally have been discovered during the evening, but elsewhere both species would have been thought absent unless the trees bordering the route had been examined. During the evenings *G. brevipalpis*, *G. pallidipes* and *G. morsitans* hovered just above the ground. The latter was often captured hovering in this way near the hour of sunset, and seemed to act thus before disappearing to rest. Still, it did not hover in exactly the same way as did *G. brevipalpis* and *G. pallidipes*, as on being alarmed it flew away in the ordinary manner. Until 4 o'clock in the afternoon, only *G. morsitans* was seen on the wing; between 4 and 5 p.m. *G. morsitans* and *G. pallidipes* were in evidence, and from 5 to 6 p.m. numerous *G. brevipalpis* were hovering near the ground. On the succeeding days, according to whether it was nearer 5 p.m. or 6 p.m., *G. morsitans* and *G. pallidipes* were still present or had disappeared, but among the *G. brevipalpis* hovering over the path, occasional *G. morsitans* were to be seen. This was

the general rule, but on many occasions *G. pallidipes* was observed earlier, sometimes as early as 3 p.m. Further, several *G. pallidipes* and *G. brevipalpis* were collected by my boys during the morning; these were not taken on trees, etc., but were more or less active. Probably this was due to the narrowness of the road, and the consequent disturbance of the resting flies by our passage. We see, therefore, that *G. morsitans*, *G. brevipalpis* and *G. pallidipes* are not limited to isolated areas or to particular places (fly belts), but occur widely distributed over large stretches of country.

Twenty-first Observation

The preceding observations have been limited almost entirely to the immediate neighbourhood of the paths, and do not extend to the interior of the forest or park where roads do not exist. The presence and habits of tsetse-flies in such places are intimately connected with the relation that exists between these flies and game—a question which has given rise to much controversy. That tsetses, like other blood-sucking diptera, bite animals is evident, but this does not mean that they feed exclusively upon them. It means still less that the existence of tsetse-flies depends solely on the presence of game, and that the absence of the latter will cause the disappearance of the fly. Wherever tsetse-flies are found, game occurs, but there are vast regions where game abounds and where there are no tsetses whatever. This is notably the case in the open country (savanes herbeuses ou peu boisées). If tsetses occur only where game exists, then they will be found everywhere throughout the Central African Colonies. It is much more logical to say that tsetses exist only where there are trees, and the objection that there are many wooded regions without tsetse-flies means nothing. It is a fact, that without shade, without trees, there would be no tsetses, and the 'extermination of trees' would certainly cause a more radical disappearance of the tsetses than the extermination of game. On the other hand, it is undeniable that game attracts tsetses; but there is, so to speak, game and game—game proper and human game. *G. palpalis* is said to be particularly attracted by human blood and to have special haunts in spots frequently visited by man, e.g. fords, places for drawing water, etc. But both *G. morsitans* and *G. palpalis* feed as much upon human blood as upon that of other animals, and the same

holds good for the other species of tsetse flies, and notably for the three species with which we are dealing. The difference between the road and the rest of the bush, from the point of view of host, is of importance, but this difference is great or negligible according to the host itself. For the human host the difference is of a capital nature, since the paths only are used by man; but for game—antelopes, buffalo, etc.—the paths are of little importance, since, although they may use them occasionally, they make their own tracks, of a temporary nature, elsewhere. Thus, if the human host is the chief one, the flies should occur almost exclusively near the roads and paths, while if the other animals are more important in this respect the flies should occur in almost any place, irrespective of roads. I have tried several times to establish an analogy between the paths and the bush, and will only state here that *G. brevipalpis*, *G. fusca* and *G. pallidipes* are very rare away from the roads. Often at the times when *G. brevipalpis* or *G. pallidipes* were abundant on the paths no sight or sound of the flies was evident a distance of ten metres or so away. Strictly speaking, an explanation of this can be made. Other conditions equal, more *G. brevipalpis* and *G. pallidipes* are seen hovering on a wide clear road than on a narrow track, probably because these insects prefer to fly near the ground and not among the taller plants, and it may be for this reason that the flies are not seen hovering far from the roads. Similar facts relate to resting flies—the further the distance from the road the fewer the tsetses on the trees, and as a rule, at twenty metres from the path scarcely any are to be seen. Exceptions to this are so uncommon that I will only enter into the details of one case: A male *G. brevipalpis* was found (about 10 a.m.) a hundred metres from the path; the fly was gorged with fresh blood, and round about were fresh tracks of buffaloes. In *Observation No. 15* it is stated that *G. fusca* was not found at mid-day on the trees near the path, but that it was present a short distance away. They were only found, however, a short distance from the path, and deeper in the bush were entirely absent. On another occasion (*Observation 19*) *G. fusca* was found, some distance from any road, in a spot where labourers were clearing the forest, but later, several days after the natives had left, *G. fusca* was camparatively rare. Not having studied this question sufficiently, no categorical state-

ment can be made, but I would point out that *G. brevipalpis*, *G. pallidipes* and *G. fusca* remain—active or resting—almost exclusively in the neighbourhood of paths. Away from paths these species are always very uncommon—a fact which merits careful study.

Twenty-second Observation

Sometimes when making these observations *G. brevipalpis* was seen resting on the leaves of a low-growing plant (more particularly on *Amomum*, a widely distributed zingiberaceous plant called by the natives 'Matungulu') and sucking the juices. The flies thus observed were males.

Twenty-third Observation

The fact that these three species of tsetse-flies prefer the neighbourhood of the roads is due to their desire to attack passers-by. We have seen previously (*21st Observation*) that when an animal passes in the immediate vicinity, *G. brevipalpis* rouses itself even during midday. It seems probable, therefore, that the passage of many animals may induce *G. pallidipes* and *G. brevipalpis* to become active at any time of the day. Opportunities arising, advantage was taken to investigate the question. At 8.30 a.m. a number (about sixty) of goats and sheep left Katompe for Kabalo, and these we accompanied until noon. No tsetses were seen on the wing, but two ♂ *G. brevipalpis*, 1 ♂ *G. pallidipes*, and 1 ♀ *Haematopota* were captured on the animals. From this it would seem that *G. brevipalpis* and *G. pallidipes* leave their resting places even during the day in order to bite—at least if the hosts are numerous—but considering the number of hosts, the potential tsetses and the insignificant number of flies captured in four hours, the habit would appear to be weakly developed.

Twenty-fourth Observation

Had the sheep and goats mentioned above passed along the road during the times of activity of the flies (4 to 6 p.m.), the number of tsetses captured on the animals would probably have been much larger, and in order to discover if such was really the case I accompanied a herd of thirty goats along the same route on the following day. These left the station at 3.30 p.m., but although between

4 and 6 p.m. the tsetse-flies (*G. pallidipes* first and *G. brevipalpis* later) were hovering near the ground in abundance, the result was negative, as not a single specimen was seen on the animals.

Twenty-fifth Observation

The two preceding observations tend to show that *G. fusca* is but little disturbed by the passage of a number of goats and sheep, since on both occasions these animals crossed the forest belt haunted by this species. Nevertheless, I decided to control these two observations. In that part of the forest which lies between Katompe and the River Namvula *G. fusca* is particularly numerous, and my boys were able to catch several dozen on the trees per day. As I was searching (with the help of five natives) for pupae in this place, I took the opportunity of tying up a small black goat so that it would be under constant observation. This animal was watched between 6 a.m. and noon, and between 2 p.m. and 6 p.m. for two days. Towards evening *G. fusca* appeared in large numbers on the trees and lianes, and from 5 to 6 p.m. showed signs of activity, numbers flying round us trying to bite. A few rested low down on the tree trunks near the goat, but not one settled on the animal. During this time *G. brevipalpis* also made its appearance, hovering over the ground as usual, but was not attracted to the animal any more than was *G. fusca*. The goat was then taken along the route from Katompe to the River Kingoï, where *G. brevipalpis* is very abundant. On two evenings we stopped there with the goat, from 4 p.m. to 6 p.m.; and between 5 and 6 p.m. this tsetse-fly swarmed on the road. On yet another occasion the goat was led up and down the road, but not a single fly made any attempt to bite or settle on it. Moreover, none of them paid any attention to my boys whose legs and feet were bare. *G. brevipalpis*, *G. pallidipes*, and *G. fusca*, therefore, are much less voracious than *G. morsitans* and *G. palpalis*, and the first two species named are still less so during their hours of activity.

Twenty-sixth Observation

One morning (about 6.30) when near the edge of the forest a female *G. brevipalpis* settled on my hat. Another time, when returning from an excursion about 6.30 p.m., a *G. brevipalpis* darted

straight at me from a tree and bit my hand before I had time to catch it; this was also a female. Six similar attacks (two in the mornings and four in the evenings) by this species were noted, and in all cases the individuals responsible were females. Females of *G. brevipalpis* scarcely ever hover above the ground in the characteristic manner of the males, and are less commonly found resting, but are much more voracious and attack more frequently. Females of this species are easily distinguished from the males by their greater bulk and especially by their greater length.

Twenty-seventh Observation

G. palpalis is said to prefer the darker colours, but although I have given this question considerable attention in respect of *G. brevipalpis*, I have not noticed it exhibit any particular preference for black. That my boys were attacked more often than myself was due to their being more in evidence and to their partial nudity. In this regard, however, the behaviour of the flies on one of my excursions struck me particularly. About 5.30 p.m., at a spot where many *G. brevipalpis* were active, I noticed the boys' hats on the ground; one of these was black and the other grey. I placed my white helmet near these, and immediately after saw a *G. brevipalpis* settle on it; in fact, a continual stream of flies flew to and from it, but not one was seen to rest on the other hats. This would tend to show that *G. brevipalpis* prefers *light* colours, and perhaps it is due to this that the fly is more numerous on a wide, clear road than on a narrow path overgrown with herbage. Consequently, had I employed a white instead of a black goat (see *Observation 25*), the flies might have been attracted in greater numbers.

Twenty-eighth Observation

I visited the forest belt on the Kabalo route at 5 p.m. Numerous *G. brevipalpis* were hovering over the ground, and many *G. fusca* as well as *G. brevipalpis* were resting on the trees along the path. *G. brevipalpis* is always more numerous at the edge of the forest, and strangely enough, instead of hovering near the ground exclusively, swarms flew round and overhead, making a considerable noise, but not attempting to bite. I note this unique occurrence in order to show that the habits of tsetse-flies are not strictly defined;

if, then, *G. brevipalpis* is seen flying high and in large numbers, it should be remembered that such an incident is exceptional, and not the rule.

Twenty-ninth Observation

During the expedition to Kabalo no *G. fusca* were seen, and I returned to Katompe no further advanced in my knowledge of the habits of this fly. In the forest and forest belts around Katompe *G. fusca* was common; its resting places were known, as also was the fact that occasional flies roused themselves throughout the day in order to bite. Preference for such attacks seemed to be given to the early morning and late evening, but the great majority of the flies remained in hiding from sunrise to sunset. Although it seemed strange to me that *G. fusca* might possibly pass its whole existence on the trees, I was about to give up the search for its definite active period when chance helped me. Having found, when travelling to Kabalo, some interesting mosquitos in the village of Kabumba (three hours from Katompe), I sent my boys to collect larvae. Being delayed in the village, the boys only left Kabumba at 5 p.m., and did not reach Katompe till after 8 p.m. In giving me an account of their mission they told me that they reached the forest belt (an hour's walk from Katompe) some time after sunset; night had already fallen, and it was so dark that they could scarcely advance. For an hour they had neither heard nor seen a tsetse, but on entering the forest they were suddenly attacked by these flies, which tried to bite them on the uncovered part of their bodies, particularly the legs. Most of the flies were captured and placed in tubes. After leaving the forest belt the attacks stopped abruptly and no more tsetses were felt until a few minutes before reaching Katompe, i.e. at the forest belt which immediately surrounds the station. Here they caught two. On giving me the tube containing the captured flies, my chief boy (who is perfectly acquainted with the morphological and biological differences between the various tsetses although not conversant with their scientific names) told me that, being without a light, he was unable to make an examination but that he thought the captured flies were those 'which had a long beak,' because the noise of their flight was much more silent than that of the 'short-beaked' tsetse. He was quite correct, as the tube

contained fifteen specimens of *G. fusca* (males and females), and no other species was present. *G. fusca*, therefore, rests throughout the whole day and only becomes active at night. So that, on one hand, we have *G. brevipalpis* with crepuscular habits, and on the other *G. fusca* with strictly nocturnal habits. But this conclusion is based on a single observation and requires verification.

Thirtieth Observation

For this purpose the forest belt referred to was visited the next day, and was reached about 4 p.m. Soon *G. brevipalpis* was seen on the road and several *G. fusca* on the trees. The sun set towards 6 o'clock; at 6.15 p.m. no sign of *G. brevipalpis* remained on that portion of the route traversing the forest, where it was getting quite dark, but at the edge of the forest it only disappeared at 6.30 p.m. By this time it was so dark in the forest that even the trees could not be seen; at 7 p.m. (an hour after sunset, and half an hour after the disappearance of the last *G. brevipalpis*), we commenced our return journey through the forest. Night had now completely fallen and there was no moon. We reached the opposite edge of belt without hearing or feeling anything whatever, but here one of the boys caught two flies on his arm. I lit a lamp in order to see the captures, and we were immediately attacked by several tsetses, one of which bit a boy in the leg; two flies were caught, and both proved to be *G. fusca*. The light was then extinguished and we continued our journey. After the forest belt the route crosses thin park country (parc dégénéré), the haunt of *G. brevipalpis* and *G. pallidipes* but not of *G. fusca*. Here, we heard almost continually the humming of insects—undoubtedly tsetse-flies—the noise being more pronounced when passing groups of trees. As *G. fusca* does not occur in this type of country, a light was obtained in order to discover the identity of the flies. First, a specimen of *G. pallidipes* (gorged with blood) was caught, and then a *G. brevipalpis*. The light was put out once more, but the humming continued until we reached a clearing where no tsetses existed. When crossing the belt of forest (8.30 p.m.) in the immediate vicinity of Katompe, scarcely any noise was detected, but a *G. fusca* was captured on a boy's arm. The more or less successful result obtained from this observation on *G. fusca* is in part vitiated by the introduction of artificial light.

Thirty-first Observation

I determined, therefore, to make a similar investigation without using light at all. We entered the forest belt at 7 p.m. and were attacked by numerous tsetses. My boys captured fifteen in fifteen minutes (the time required to cross the forest belt); two flies were also captured a certain distance away from the forest, but these had evidently followed us. Later on we heard the same noise as on the previous evening, but did not catch any flies until we reached the Katompe forest where three were taken. On returning home, the specimens collected were examined, and were all found to be *G. fusca*. All these specimens (twenty), as well as all those collected on the previous evening, were captured by my boys—I did not catch one. This was due to the fact that the boys' legs and arms were bare and consequently they could feel the tsetses settle on them much better than I. In fact, the results of these two observations would have been negative without their help.

Thirty-second Observation

I decided to make further observations on the lines indicated above but to carry them out in the immediate neighbourhood of Katompe since the roads here, and especially those leading across the forest to the Rivers Namvula and Kingoï, were haunts of the fly and entirely suitable for the purpose. For six days in succession, between 7 and 8 p.m., visits were paid to the forest, and each time many specimens of *G. fusca* were caught preparing to bite, or in act of biting, the boys. The numbers collected varied—one night five were caught, another night eight, and so on; more were obtained on cool than on warm evenings, and it was again noticed that the noise made by *G. fusca* when flying was much less than that made by *G. brevipalpis*. By following on several occasions the direction from which this noise came, my belief that *G. fusca* left a neighbouring tree in order to attack us was confirmed. Not once were we attacked at this time by *G. brevipalpis*, in spite of its abundance in this spot. On one occasion, when part of the journey was made with a light, two *G. brevipalpis* came flying round us, but departed without even resting upon us. The question of the length of time that *G. fusca* is active, whether it is so throughout the night or at least until midnight, could not be investigated owing to the termination of

my stay at Katompe. This tsetse-fly, however, as shown above, is always active till 8.30 p.m. (about two hours after sunset), but later than this the forest was only visited on one ocasion (at 9.30 p.m.), and then there was no sign of the fly and no attacks were made upon us. Apart from rare cases then, *G. fusca* is only active at night, or at least very late in the evening—one or two hours after sunset—and therefore may well be said to possess nocturnal habits in contradistinction to the crepuscular habits of *G. brevipalpis*. There is also another great difference between the habits of these two species—while *G. brevipalpis* hovers over the paths and very rarely attacks passers-by during its period of activity, *G. fusca* remains at rest on the trees and only leaves them in order to obtain blood. *G. fusca*, therefore, would also seem much more voracious than *G. brevipalpis*.

Thirty-third Observation

After having left Katompe, I went to Kongolo (see map). In general, this route recalled that from Katompe to Kabalo, and as in the case of the latter route, *G. brevipalpis*, *G. pallidipes* and *G. morsitans* occurred, but not *G. fusca*. But later, when travelling from Kongolo to Kabinda, I found *G. fusca* in several places where there were wide belts of forest. On this journey we reached the stream Bululu (near the village Kikamba) at 10 a.m. This stream is bordered with a belt of forest (galerie forestière) several hundred metres long. A halt was made here, and after a few minutes *G. palpalis* was observed; my carriers captured a few specimens and then no more were seen. An examination of the trees and lianes, however, revealed the presence of both *G. brevipalpis* and *G. fusca*. The next day (also about 10 a.m.) we reached a small stream—the Kina—where there was a wide tract of dense forest. Several *G. palpalis* were found, and a rapid examination of the trees enabled us to find *G. brevipalpis* and *G. fusca*. I stopped that night at the village Kiumbi, about forty minutes from the Kina, and taking the opportunity returned to the Kina after sunset. On the way there I met my carriers who were returning with several hundred *G. brevipalpis* collected in the Kina forest, but among these there was not a single specimen of *G. fusca*. Two of the porters returned with me and my boys to the forest, and in half an hour—from 7 to 7.30 p.m.—the four of them captured on themselves

forty-eight examples of *G. fusca* (36 ♂s and 12 ♀s). No *G. brevipalpis* were taken during this time, although the noise of their flight was sometimes heard. This observation again supports those preceding, and shows very distinctly the difference between the habits of *G. brevipalpis* and *G. fusca*.

Thirty-fourth Observation

Between the villages Kifukutu and Mwepo (on the Kongolo-Kabinda route, west of the River Lomami) the whole route of about twelve kilometres is well, but not uniformly, wooded. This stretch of the route crosses four streams with wide forest galeries, and between these streams are wide belts of park and wooded savannah. These three types of vegetation are clearly defined and arranged according to their densities—forest, park, wooded savannah, park, forest, park, wooded savannah, etc., etc. Along the whole twelve kilometres of road, between 6 a.m. and 10 a.m. only one specimen (♀) of *G. brevipalpis* was taken on the wing; it was in the act of biting one of the carriers. Twenty-two examples of *G. fusca*, however, were collected from the trees in these wide tracts of forest, but in no other parts of the route. *G. brevipalpis* was less exclusive in this respect and was found on trees along the whole route. Among twenty *G. brevipalpis* captured in different places on the trees were two females, one of which was in coitu. *G. pallidipes* completely disappeared in the vicinity of Mwepo (its western limit), and throughout the whole route in question was only seen three times—once, hovering over the path, and twice resting on tree trunks, and on all three occasions was found in the country intervening between the forest belts. The observations made between Mwepo and Kifukutu thus confirmed those made at Katompe on the relation of the different species of tsetses to the different types of arborescent vegetation.

Thirty-fifth Observation

By examining the map of the route from Kongolo to Kabinda, it will be seen that at the passage of two streams between the villages Sulu and Kifulu (to the east of the River Lomani) *G. brevipalpis* and *G. pallidipes* were found. From Mwepo to Kikumbi (between Rivers Lomani and Lukasi) *G. brevipalpis* was present on all the

river crossings, but no *G. pallidipes* occurred. Beyond the River Lukasi neither *G. brevipalpis* nor *G. pallidipes* were seen at any of the numerous passages over the streams. The country, in all the places mentioned, is more or less the same—grassy or sparsely wooded savannah and, usually, narrow 'forest galeries' along the streams and rivers. The country being similar throughout, why should there be this difference regarding these tsetse-flies? The reason is that *G. pallidipes* does not occur westward of Mwepo (at least it was no longer seen), while the area of dispersion of *G. brevipalpis* does not extend further than the River Lukasi. Therefore, the principles maintained above relating to the dependence of a particular species of *Glossina* on the type of vegetation only holds in those regions where this species of tsetse occurs. Further, even in these regions the principle is only of value in positive cases, e.g. in the regions where *G. fusca* exists it is only found in moderately wide belts of forest and does not occur elsewhere, but, in the same region, there may be wide forest belts where *G. fusca* does not occur.

Thirty-sixth Observation

The result of observations on *G. pallidipes* at Katompe and along the roads leading from Katompe to Kabalo, and from Katompe to Kongolo, is that from the point of view of habitat, this species resembles *G. morsitans* in that it does not inhabit the forest. But this result is only applicable to wooded regions, to regions where vast stretches of park, wooded savannah (savane bien boisée), and wide tracks of forest occur. In open country (savane herbeuse), where trees only occur in the low-lying parts, forming small islets of arborescent vegetation near streams and rivers, an occasional *G. pallidipes* may be found. And this although these islets resemble forest rather than park-land. Still it would be an exaggeration to say that *G. pallidipes* cannot exist among dense vegetation. When the belt is wide and dense *G. pallidipes* will not be found in the interior, but may occur at the edges. It may be said, therefore, that *G. pallidipes* occurs (in the same places, and even resting on the same trees) with *G. morsitans* and *G. brevipalpis*, but not with *G. fusca*; and that *G. fusca* occurs with *G. brevipalpis* and *G. palpalis*, but not with *G. morsitans* or *G. pallidipes*.

These statements may be summarised in the following schematic manner:—

1. Forest (or forest belt) - - - - - { *G. palpalis*, *G. fusca*, *G. brevipalpis*

2. Park (or wood) - - - - - - { *G. morsitans*, *G. brevipalpis*, *G. pallidipes*

3. Wooded savannah (savane trés boisée) - { *G. brevipalpis*, *G. pallidipes*

4. Islets of mixed vegetation
   - without water { *G. brevipalpis*, *G. pallidipes*
   - with water { *G. brevipalpis*, *G. pallidipes*, *G. palpalis*

Thirty-seventh Observation

Although this article is not concerned with *G. palpalis*, remarks on some little known but important facts concerning this species may perhaps be made. At Katompe, on the River Namvula, *G. palpalis* was extremely rare, so that no records regarding the species in this region will be made. In the 'forest galeries' of the streams Bululu and Kina *G. fusca* was common (*23rd Observation*), but *G. palpalis* was also found resting on trees and lianes. One specimen was resting on the under-surface of a liane stretching horizontally above the stream about a metre from the surface; a second was found on a more or less vertical liane, and a third on a tree trunk. The last two flies were resting *with their heads directed upwards*.

Thirty-eighth Observation

Some days after the above observation was made I crossed (at 7 a.m.) the Kafulungoi (on the Kongolo-Kabinda route between the village Tanganika and the River Lukasi), a small clear flowing stream situated in a rather wide marshy forest belt. The ford being comparatively near the mouth of the stream, search was made for *G. palpalis*, for although I expected to find it here, I did not believe it would be active owing to rain during the night having made the

morning cold and damp. Within five minutes three torpid specimens of this tsetse were found on the trees—all resting with their heads pointing upwards.

The successful detection of *G. palpalis* during the time it is in hiding, e.g. in the early morning after heavy rain, can be made by examining tree trunks and lianes, and naturally is of considerable importance if the object be to prepare a map showing distribution of this species. But unfortunately this method is not always practicable, as such a search requires considerable experience and keen vision. For instance, even in spots where *G. brevipalpis* swarms during the evening, an hour's search of the trees, etc., by myself and my boys has often resulted in only two or three flies being found. Consequently, it is very probable that no success will often attend an examination made in places where tsetse-flies are rare as is frequently the case with *G. palpalis* at the crossing of small streams. Obviously then, if such remarks relate to *G. brevipalpis*, which is only active for an hour or two in the evenings, to *G. pallidipes* which is usually only active for a couple of hours in the afternoon, and to *G. fusca* which, with rare exceptions, remains hidden all day, then recourse to other means is necessary to confirm the presence of these tsetses. But *G. palpalis* is active *almost* all day, so that usually it is not difficult to determine its presence. In places where it is so scarce that the chance of seeing it is small, the chance of finding it on the trees is smaller still, and thus the detection of *G. palpalis* by the examination of trees, etc., has a practical value only in spots where it is moderately abundant, or where, owing to certain circumstances (the earliness of the hour or coldness and dampness due to heavy rain), it is not active for the time being.

Thirty-ninth Observation

The manner of resting adopted by the species of *Glossina* referred to in this paper is shown in the following table:—

1. Head downwards	-	*G. brevipalpis* *G. fusca*
2. Head upwards	- -	*G. palpalis* *G. morsitans*
3. Position variable	- -	*G. pallidipes* (the head more often points upwards than downwards)

It may be said, therefore, that the larger species rest with their heads downwards and the smaller species with their heads upwards, but that *G. pallidipes*, which is intermediate in size and habits between *G. morsitans* and *G. brevipalpis*, holds an intermediate position in this respect. Or we may say that tsetse-flies with diurnal habits rest with their heads upwards, and those with crepuscular or nocturnal habits, with their heads downwards, while *G. pallidipes*, being more or less intermediate regarding its time of activity, is also intermediate with regard to its resting position. Here it may be mentioned, however, that when confined in large glass jars *G. fusca* and *G. brevipalpis* rest with their heads directed upwards.

Fortieth Observation

Although I had no intention of breeding tsetses during my stay at Katompe, I sometimes enclosed gravid females of *G. fusca*, *G. brevipalpis* and *G. pallidipes* in glass jars covered with gauze and half filled with sand. On two occasions all the caged tsetses were devoured by small lizards which broke through the gauze and were found later in the jars. Little success was obtained with those remaining. The majority died one or two days after their capture, and at the end of three days not one was living. This rapid mortality was due rather to external injuries than to their confinement. I did not observe the captive tsetses (at least those in the glass jars) to show increased activity at any particular time of the day or night. They flew about continually, and were constantly dashing themselves against the sides of the jars. The feebler they became the more tranquil they were; during the night they were much quieter, but roused themselves when artificial light was introduced. Most of the larvae were aborted; the majority were dead and very small. The few living larvae which were obtained soon died—some without even penetrating into the sand—and shrivelled up before pupation.

Forty-first Observation

A ten days' search was made during March for the pupae of *G. brevipalpis*, *G. fusca* and *G. pallidipes*. The time was badly chosen, as it rained almost every day and the ground was very wet. Before leaving Katompe I was obliged to discontinue these researches,

but fortunately not before some slight measure of success had been obtained. Knowing the resting-places of these flies, it was considered probable that the larvae bury themselves in the ground below the trees, and accordingly search was made in such spots in the haunts of the species in question. The dead leaves were examined first, then the humus, and lastly the ground itself. The pupae of other insects (*Coleoptera* and *Muscidae*) were numerous, but a certain number of pupae of *G. fusca* and *G. brevipalpis* were discovered. Most of these were empty, but ten were whole (two, I believe, were those of *G. fusca*), from three of which males of *G. brevipalpis* hatched. All the pupae were found in the ground from 3 to 5 cms. below the humus layer; some were lying between the roots of the trees, some a certain distance away, but always in the shade. I hope to confirm these researches later.

That pupae of *G. brevipalpis* are capable of resisting external injuries and shocks was well shown during my journey from Katompe to Kabinda. The morning after my departure from Katompe (April 2nd) I saw a female *G. brevipalpis* (with greatly distended abdomen) resting on a tree trunk. On being captured it immediately deposited a large white larva, which at once commenced to move about my hand. The larva was placed in a tube half filled with sand, and quickly buried itself. In the afternoon I examined the tube and found there a black and motionless pupa. The tube was then put into a trunk and wedged between various articles to prevent breakage and to ensure it maintaining a vertical position. I arrived at Kabinda thirty-four days later (May 15th), after travelling 450 kilometres on foot, and naturally during such a journey the trunk had been considerably shaken. The next day (May 16th) I examined the pupa, which I believed to be dead and intended adding to my collection of pupae. However, I was prevented from doing so just then, and returning to the tube the next morning with the same intention was much surprised to see therein a living male *G. brevipalpis*. The duration of the pupal stage in this case was thirty-six days.

SUMMARY AND CONCLUSIONS

1. The five important tsetse-flies—*G. brevipalpis*, *G. fusca*, *G. pallidipes*, *G. morsitans* and *G. palpalis*—select tree trunks, the larger branches of trees and lianes for resting purposes.

2. In regions where they exist, *G. brevipalpis* and *G. pallidipes* are not restricted to limited areas or belts, but, like *G. morsitans*, are found uninterruptedly—except in large clearings—over vast stretches of country.

3. The habits of *G. pallidipes* are intermediate between those of *G. brevipalpis* and *G. morsitans*. Like *G. brevipalpis*, *G. pallidipes* hovers over the ground when active and is usually so only at certain fixed times, especially between 3 p.m. and 5 p.m. (4 to 6 p.m. with *G. brevipalpis*), the maximum activity being reached about 4 p.m. The habits of *G. pallidipes*, however, are less well defined than those of *G. brevipalpis*, and it not only appears and bites more often than the latter, but is not uncommonly seen on the wing in very small numbers throughout the whole afternoon and occasionally in the forenoon. Of the *G. pallidipes* captured when active, only about 15 per cent. were females.

4. In those regions where *G. brevipalpis* occurs it accommodates itself to all types of arborescent vegetation—forest, parkland and wooded savannah, but *G. pallidipes*, like *G. morsitans*, does not inhabit the forest.

5. *G. fusca* occurs only in one kind of arborescent vegetation—forest. As the region where these observations were made (northern Katanga, notably the districts between the River Lualaba and the upper Lomami River) consists of parkland and savannah, the forest only being represented, usually along rivers and streams, by belts of varying width, it is evident that in this region *G. fusca* only occurs in limited and somewhat restricted areas. Further, these areas are still more restricted owing to the fact that this species only inhabits moderately dense forest belts of a certain width (200 to 300 metres). But contrary to what has been thought so far, where *G. fusca* does occur it is not at all uncommon, and sometimes it is quite abundant. However, it has peculiar habits—it does not fly during the day like *G. morsitans* and *G. palpalis*, it does not hover over the ground at

definite times like *G. brevipalpis* and *G. pallidipes*, but always remains motionless on tree trunks and lianes. Occasional specimens, usually one or two, are sometimes attracted by men and animals passing by. These may make their appearance at any time of the day, but prefer the cooler hours, e.g. early in the morning or more often late in the evening. But *G. fusca* also has a definite period of activity, namely from 7 to 8 p.m., or one to two hours after sunset, and if a haunt of the fly be passed during this time, numerous attacks are sure to be made.

6. Females of *G. fusca*, unlike those of *G. brevipalpis* and *G. pallidipes*, are commonly found, and form nearly 50 per cent. of the specimens captured—whether the flies be taken on the wing or resting on trees, etc.

7. Since forest belts usually occur near water, it is in the neighbourhood of the latter that *G. fusca* is generally found, but *G. fusca* also occurs in forest belts where there is no water in the immediate vicinity, and may even be found in forest a few kilometres distant from the nearest water. In this respect, therefore, *G. fusca* is unlike *G. palpalis*, but resembles *G. pallidipes*, *G. brevipalpis* and *G. morsitans*.

8. The haunts of *G. brevipalpis*, *G. pallidipes* and *G. fusca* are situated almost exclusively along the roads and paths.

Before concluding this article, I may mention that for several years I have been preparing a map showing the distribution of tsetse-flies in certain regions (the Lomami district and neighbouring areas) of Northern Katanga. I have already published several maps, but it is now evident from the above observations that these are incomplete. Even the map which is attached to this paper is exact (or so, I hope) only on and after Katompe, for before that, during the outward journey from Kabinda to Katompe via Kisengwa, I was unacquainted with the particular points given above.

Lastly, certain figures which I have collected regarding the proportions of the sexes existing among different batches of tsetse-flies captured round Katompe and elsewhere may be of interest:—

1. Of 165 specimens of *G. fusca* captured at Katompe in February (a few of which were taken on the wing), 88 were males and 77 females.

2. Of 255 specimens of *G. fusca* captured at Katompe in March, 143 were males and 112 females.

3. Several species of tsetses captured one morning on tree trunks between Masembi and Musinga Lenge were divided as follows:—

(*a*) 27 *G. brevipalpis*, 21 males, 6 females.
(*b*) 4 *G. pallidipes*, 3 „ 1 „
(*c*) 7 *G. fusca*, 4 „ 3 „

4. Of 344 *G. pallidipes* captured at Katompe from February to March, 281 were males and 63 females.

5. Of 237 *G. pallidipes* captured on the Katompe-Kabalo route in February, 193 were males and 44 females.

6. Of 509 *G. morsitans* captured on the same route during the same journey, 304 were males and 205 females.

7. Of some 5,000 *G. brevipalpis* captured at Katompe and elsewhere, only 50 were females, and of these about 75 per cent. were captured on tree trunks.

KABINDA,
June 30th, 1916.

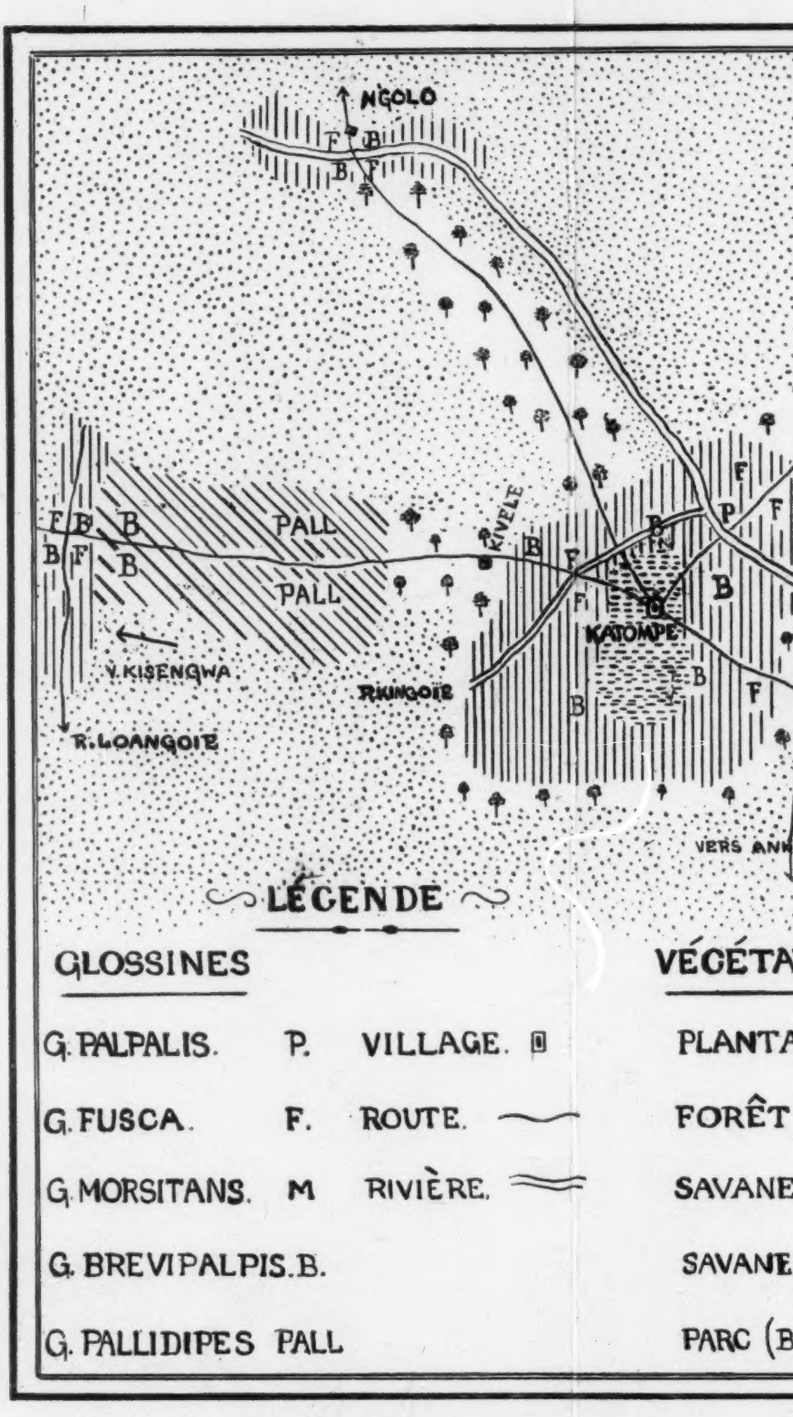
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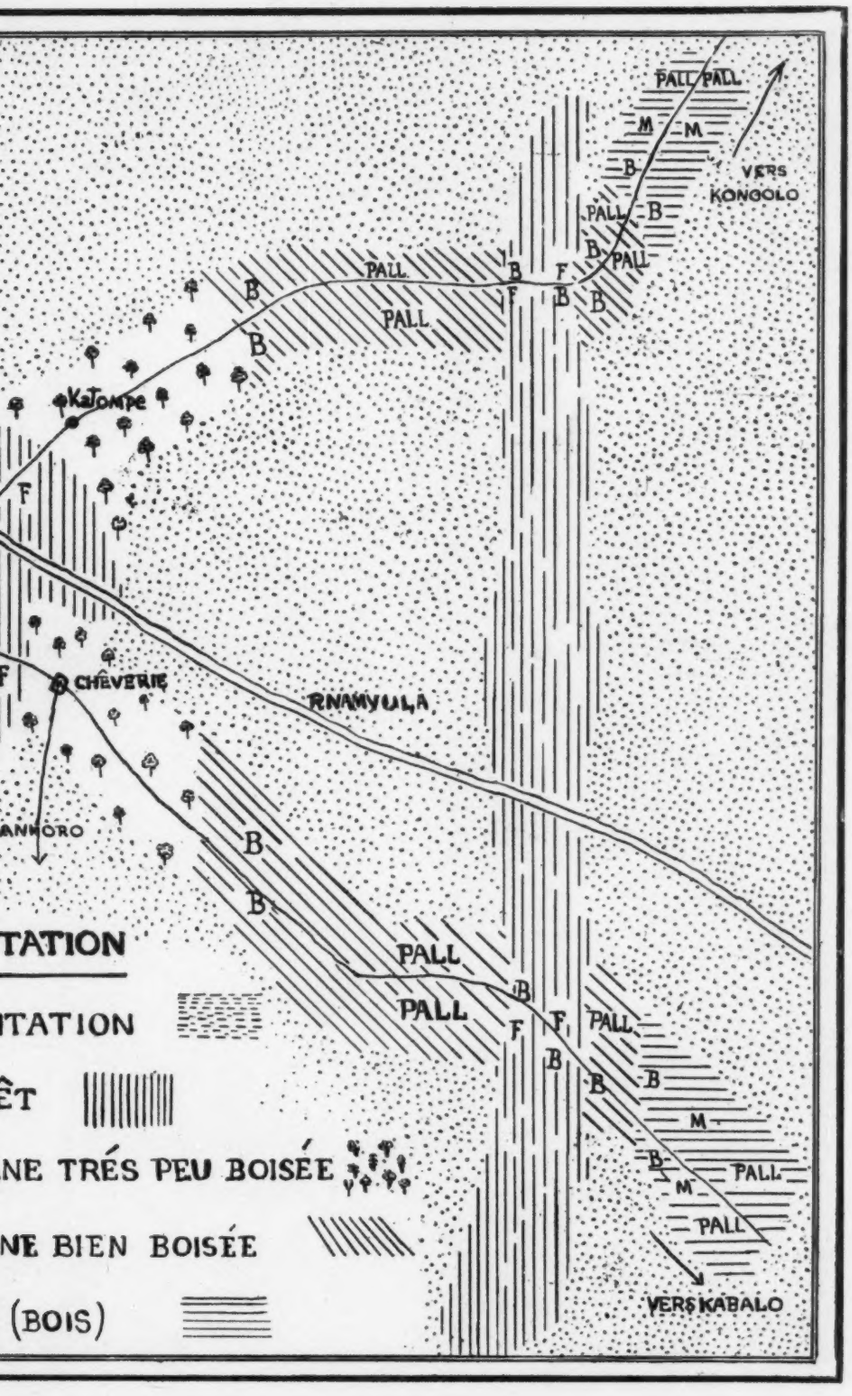
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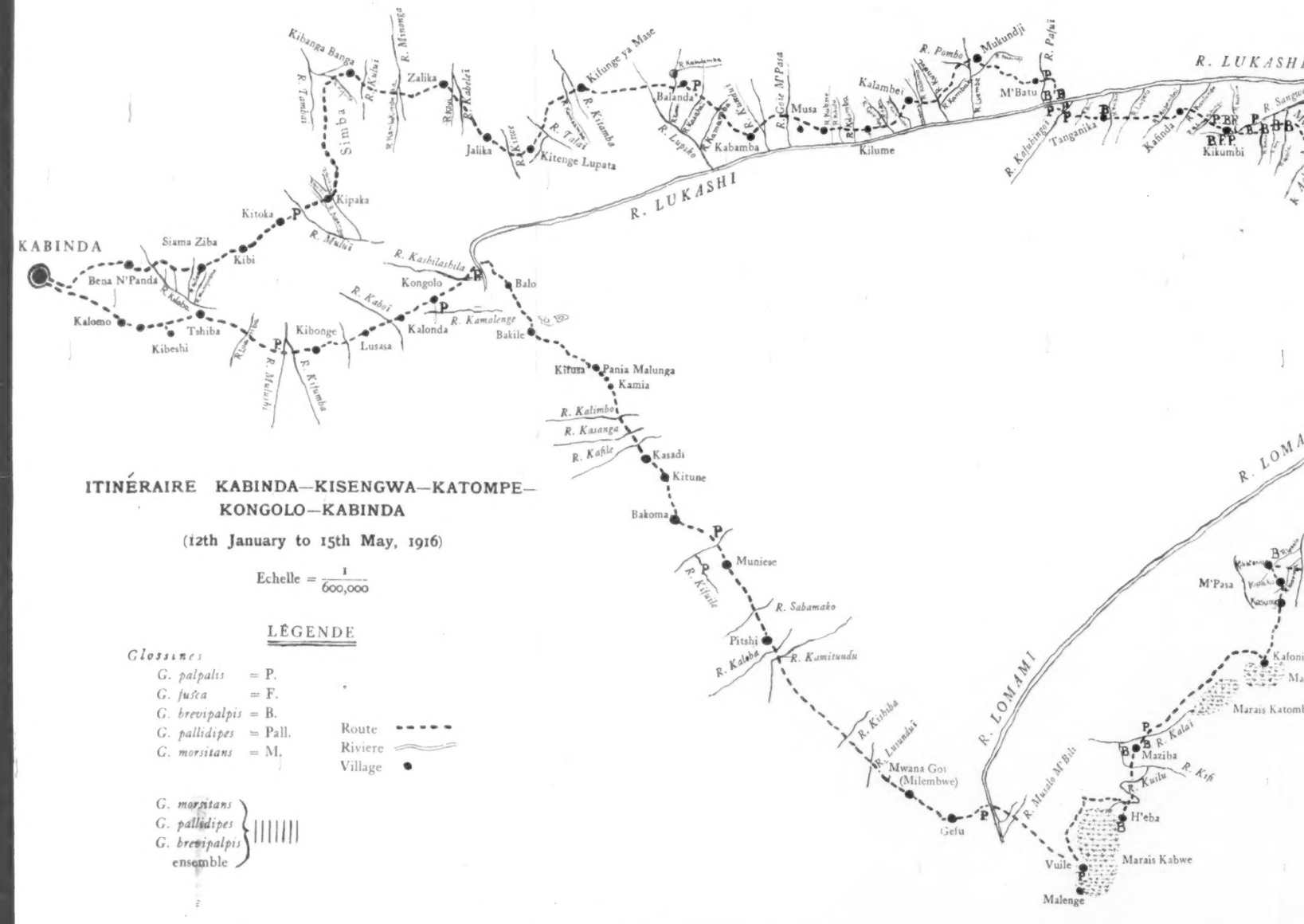
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NGOLO
PALL.
PALL.
KIVELE
KATOMPE
V. KISENGWA
RIKINGOIE
R. LOANGOIE
VERS ANK
LÉGENDE
GLOSSINES
G. PALPALIS. P.
G. FUSCA. F.
G. MORSITANS. M
G. BREVIPALPIS. B.
G. PALLIDIPES PALL
VILLAGE.
ROUTE.
RIVIÈRE.
VÉGÉTA
PLANTA
FORÊT
SAVANE
SAVANE
PARC (B


PALL
M
B
F
VERS
KONGOLO
KATONDE
CHÈVERIE
RNAMYULA
ANHORO
TATION
ITATION
ÊT
NE TRÉS PEU BOISÉE
NE BIEN BOISÉE
(BOIS)
VERS KABALO



ITINÉRAIRE KABINDA—KISENGWA—KATOMPE—KONGOLO—KABINDA

(12th January to 15th May, 1916)

Echelle = $\frac{1}{600,000}$

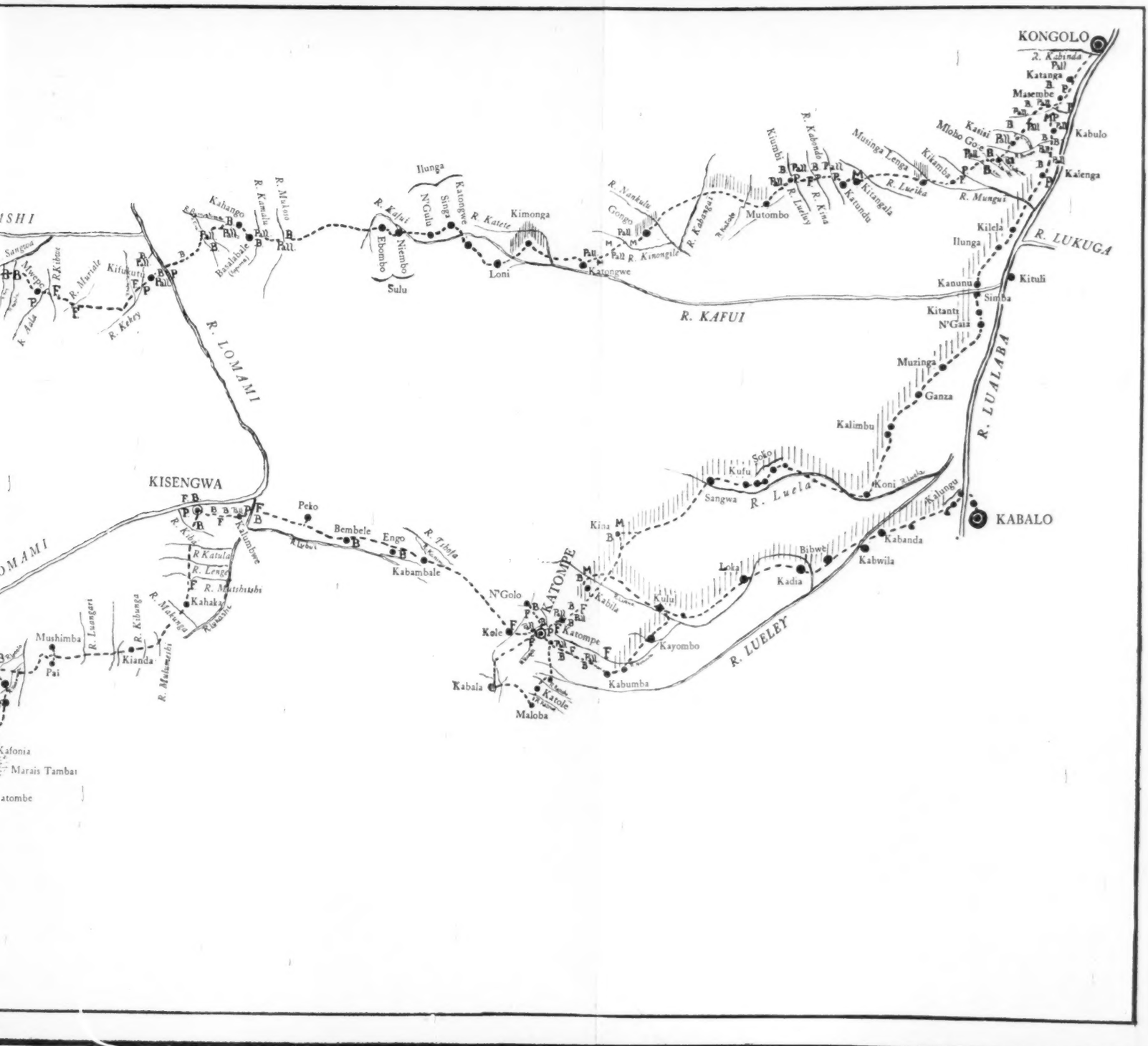
LÉGENDE

Glossines

G. palpalis = P.
G. fusca = F.
G. brevipalpis = B.
G. pallidipes = Pall.
G. morsitans = M.

Route
Riviere
Village

G. morsitans
G. pallidipes
G. brevipalpis
ensemble


KONGOLO
R. Kabinda
Pall
Katanga
Masembe
Kabulo
Kalenga
Kiambi
Musinga Lenga
Kitamba
R. Lurika
R. Mungui
Mloho Go
Kilela
Ilunga
R. LUKUGA
Kanunu
Kituli
Simba
Kitanti
N'Gaia
R. KAFUI
Muzinga
Ganza
Kalimbu
R. LUALABA
Kufu
Soko
Sangwa
R. Luela
Koni
Kalungu
KABALO
Kabanda
Kabwila
Bibwe
Kadia
Loka
Kina
Kulu
Kayombo
R. LUELEY
Kabumba
KATOMPE
Kole
N'Golo
Kabala
Katole
Maloba
Kabila
Katompe
KISENGWA
Peko
Bembele
Engo
Kabambale
R. Tibafa
R. Kalumbwe
R. Katula
R. Lenge
R. Matshitshi
Kahaka
R. Mabanga
R. Kibunga
R. Luangeri
Mushimba
Pai
Kianda
R. Malumeiti
R. LOMAMI
Kahango
Basalabale
R. Kamalia
R. Makoso
Ilunga
N'Gulu
Singa
Katongwe
R. Katete
Kimonga
R. Kafui
Ebombo
Niembo
Sulu
Loni
R. Nankula
Gongo
R. Kinongale
R. Kabangala
Mutombo
Kiumbi
R. Kabondo
R. Lueley
R. Kina
Kitundu
Kitangala
Sangwa
R. Mariale
R. Kibesi
R. Kafey
Kitukuri
Kafonia
Marais Tambat
atombe

STRONGYLIDAE IN HORSES

BY

WARRINGTON YORKE

AND

J. W. S. MACFIE

From the Liverpool School of Tropical Medicine

(*Received for publication* 24 *January*, 1918)

INTRODUCTION

Through the courtesy of Major Brittlebank, A.V.C., we have had the opportunity of examining for parasitic worms a number of horses which had recently come from the United States of America and which had died shortly after arrival in this country. Infection with *Strongylidae* was, in the opinion of the veterinary surgeons, the cause of death.

On post-mortem examination large numbers of many different species of *Strongylidae* were found in the caecum and colon.

We propose in a series of notes to enumerate the species found, and to describe those which have not hitherto been recognised.

Technique. The worms, which were removed from the intestine as soon after death as possible, were fixed in hot 70 per cent. alcohol. They were subsequently slowly impregnated with glycerine by placing them in a solution of 70 per cent. alcohol containing 5 per cent. glycerine and allowing the alcohol to evaporate off at 37° C.; when impregnation was complete they were mounted in glycerine jelly (*vide* Looss, 1901).

In the following descriptions, terms such as 'long' or 'short' having a purely relative value are, so far as possible, replaced or supplemented by figures obtained by actual measurement. For example, it is of little value to know that the oesophagus or dorsal

lobe of the bursa is 'long' or 'short.' It is of greater assistance to know that these structures are of a certain definite length, but even this information hardly conveys any accurate impression unless it is associated with the length of the worm. In the following notes, therefore, we propose to express such lengths as that of the oesophagus or that of the main trunk of the posterior ray of the bursa, measured from the tip to the point of origin of the postero-external ray, as ratios of the total length of the worm.

1. *CYLICOSTOMUM LONGIBURSATUM* sp. n.

SIZE AND SHAPE. A small slender species of the GENUS *Cylicostomum*; on account of the remarkably long dorsal lobe of the bursa, the males and females are of about the same length. Ten males and ten females were measured. The males were from 4·8 to 5·5 mm. in length, average 5·1 mm.; the females were from 4·7 to 5·7 mm., average 5·1 mm.; the greatest breadth, in those worms which were properly orientated (lying on dorsal surface), averaged, males 182μ, females 232μ.

HEAD. The head is separated from the body by a slight neck.

Mouth collar. Marked off from the rest of the skin by a sharp constriction. The mouth is circular in transverse section.

Head papillae. Sub-median, project anteriorly slightly beyond the external leaf crown; near their extremities are minute lateral notches: lateral, not projecting beyond the surface of the mouth collar.

Mouth capsule. Circular in transverse section; the walls of the mouth capsule seen in optical section are slightly kneed, they are moderately stout and diverge from before backwards, so that the cavity has the shape of a truncated cone (fig. 1). The antero-posterior diameter (i.e. the distance from the anterior to the posterior opening) of the cavity in ten worms varied from 17·5μ to 20μ, average 19μ; the lateral diameter at the anterior opening varied from 18μ to 20μ, average 19μ, and that at the posterior opening from 24μ to 28μ, average 26μ. The ratio of the lateral diameter of the anterior opening of the mouth capsule to the antero-posterior diameter is 1 to 1.

Dorsal oesophageal gutter. Projects slightly into the buccal cavity, but does not extend further than a fourth of the antero-posterior diameter.

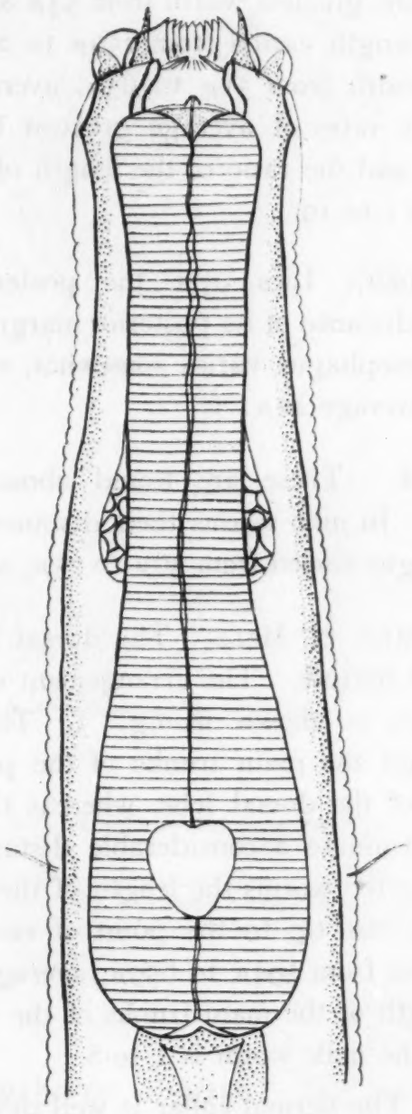


FIG. 1. *Cylicostomum longibursatum* sp. n.
Anterior extremity, ventral view, × 360.

Leaf crowns. The external leaf crown consists of eighteen long narrow elements pointed at the tips arising from the mouth collar. The internal leaf crown consists of about the same number of short

and broad elements arising near the anterior opening of the buccal cavity.

OESOPHAGUS. The length in ten males varied from 248μ to 292μ, average 271μ, and the greatest width from 53μ to 64μ, average 57μ; in ten females the length varied from 259μ to 290μ, average 270μ, and the greatest width from 51μ to 63μ, average 58μ. In both sexes, therefore, the ratio of average greatest breadth to average length is 1 to 4·7; and the ratio of the length of the oesophagus to that of the worm is 1 to 19.

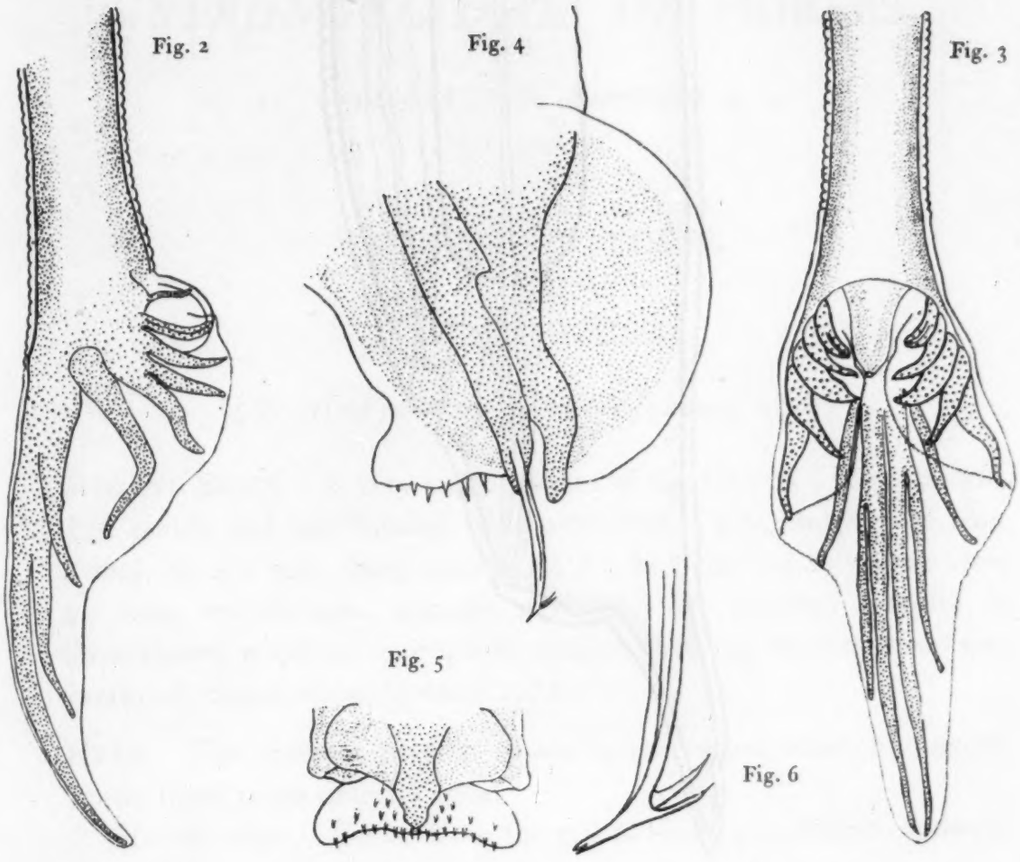
EXCRETORY BLADDER. Lies over the posterior fourth of the oesophagus. The distance of its posterior margin from the posterior extremity of the oesophagus varies somewhat, e.g. in seven worms from 26μ to 63μ, average 42μ.

CERVICAL PAPILLAE. These are found about the level of the excretory bladder. In nine worms their distance from the posterior end of the oesophagus varied from 36μ to 75μ, average 58μ.

POSTERIOR EXTREMITY OF MALE. The dorsal lobe of the bursa is strikingly long and narrow. The arrangement of the posterior rays and their branches is shown in fig. 3. The worm resembles *C. calicatum* in that the main trunks of the posterior ray extend almost to the tip of the dorsal lobe, whereas the lower or second lateral branches terminate a considerable distance from the tip of the dorsal lobe. In ten worms the length of the main trunks of the posterior ray, from the tip to the point of origin of the postero-external rays, varied from 594μ to 659μ, average 631μ. The ratio of the average length of the main trunks of the posterior ray to the average length of the male worm is 1 to 8.

Genital Cone. The dermal collar is well developed on both the dorsal and ventral surfaces of the genital cone. The genital appendages are conical when viewed laterally, but broad and united in the middle line when viewed ventrally; their ventral surface is beset with small papillae (figs. 4 and 5).

Spicules. The ends of the spicules are barbed, as shown in fig. 6.



FIGS. 2-6. *Cylicostomum longibursatum* sp. n.

Fig. 2: Posterior extremity of male, lateral view, × 90. Fig. 3: Posterior extremity of male, ventral view, × 90. Fig. 4: Genital cone and appendages, lateral view, × 360. Fig. 5: Genital appendages, ventral view, × 360. Fig. 6: Ends of the spicules, × 1360.

POSTERIOR EXTREMITY OF FEMALE. The end of the body is straight and tapers slightly. The tail, which is distinctly demarcated from the end of the body, is straight, fairly long and tapers gradually to a point (fig. 7). In ten worms the distance between the anus and vulva varied from 51μ to 73μ, average 62μ; and the distance measured straight along the middle of the tail from the tip to a line drawn horizontally through the anus varied from 95μ to 124μ, average 104μ.

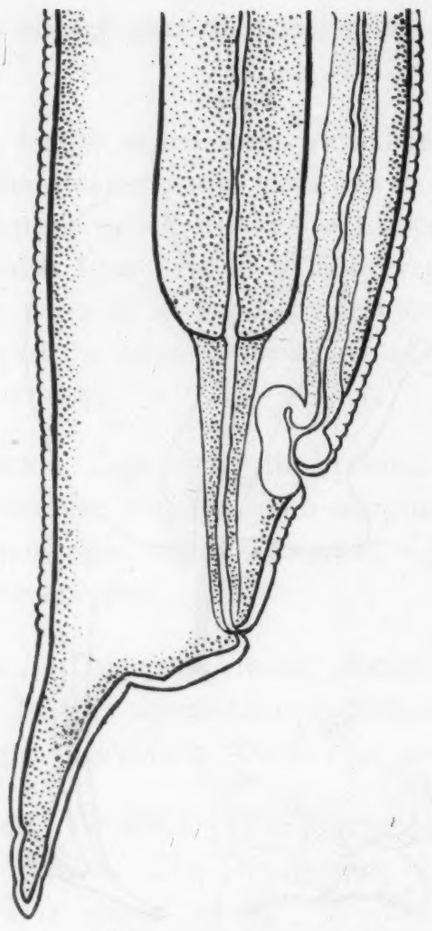


FIG. 7. *Cylicostomum longibursatum* sp. n.

Posterior extremity of female, lateral view, × 360.

DIAGNOSIS. The following are the chief diagnostic characters of this species:—

1. Very small size; average length, male and female, 5·1 mm.

2. Buccal capsule: anterior opening circular; walls, when seen in optical section, kneed; ratio of breadth at anterior opening to antero-posterior diameter 1 to 1.

3. Dorsal oesophageal gutter projects slightly into buccal capsule.

4. Dorsal lobe of bursa very long and narrow; ratio of length of posterior ray to total length of male worm 1 to 8.

5. Termination of female body and tail straight.

REFERENCE

Looss, A. (1901). The Sclerostomidae of Horses and Donkeys in Egypt. *Records of the School of Medicine, Cairo*, Vol. I.

STRONGYLIDAE IN HORSES

II. *CYLICOSTOMUM MINUTUM* sp. n.

BY

WARRINGTON YORKE

AND

J. W. S. MACFIE

(*Received for publication* 31 *January*, 1918)

SIZE AND SHAPE. A very small species of the GENUS *Cylicostomum*. Ten males and ten females were measured. The males were from 4 mm. to 4·6 mm. long, average 4·3 mm.; the females were from 4·1 mm. to 5·6 mm., average 4·9 mm.; the greatest breadth, in those worms which were properly orientated (lying on dorsal surface), averaged, males 220μ, females 228μ.

HEAD. The anterior portion of the body tapers markedly to the head; there is no definite neck.

Mouth collar. Set off from the rest of skin by a definite constriction. The mouth is circular in transverse section.

Head papillae. Submedian, long; near their extremities are minute lateral notches: lateral, scarcely projecting beyond the surface of the mouth collar.

Mouth capsule. Circular in transverse section; the walls of the mouth capsule seen in optical section are straight, they are slightly thicker posteriorly and diverge from before backwards (fig. 1). The antero-posterior diameter (i.e. the distance from the anterior to the posterior opening) of the cavity in ten worms varied from 20μ to 27μ, average 23μ; the lateral diameter at the anterior opening varied from 15μ to 19·5μ, average 16·7μ, and that at the posterior opening from 18·5μ to 22μ, average 20·2μ. The ratio of the lateral diameter of the anterior opening of the mouth capsule to the antero-posterior diameter is approximately 1 to 1·4.

Dorsal oesophageal gutter. Well developed and projects far into the mouth capsule, extending nearly to the anterior opening.

Leaf crowns. The external leaf crown consists of eight rather broad elements arising from the mouth collar. The internal leaf crown is composed of about twenty short, stout elements arising from the anterior margin of the mouth capsule.

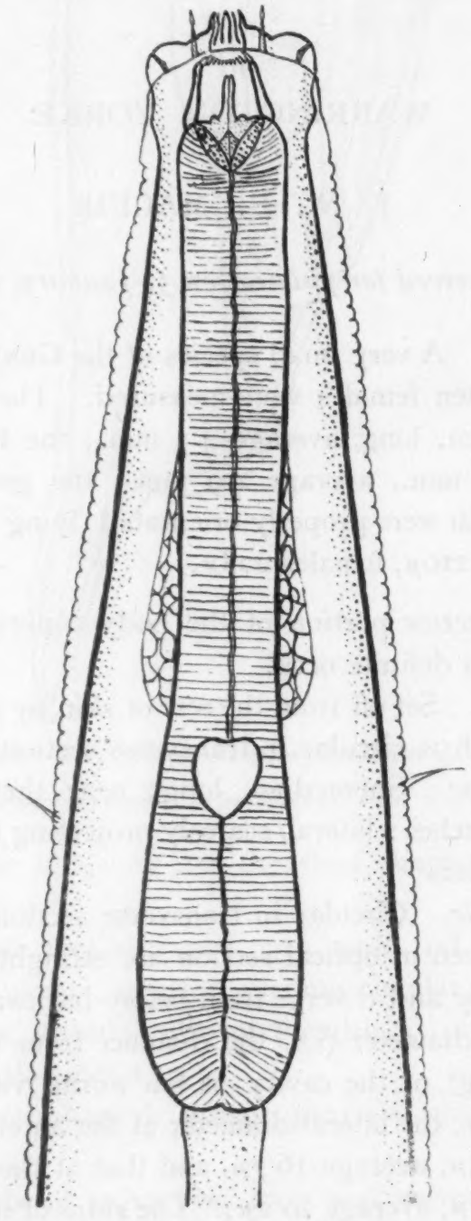


FIG. 1. *Cylicostomum minutum* sp. n.
Anterior extremity, ventral view, × 360.

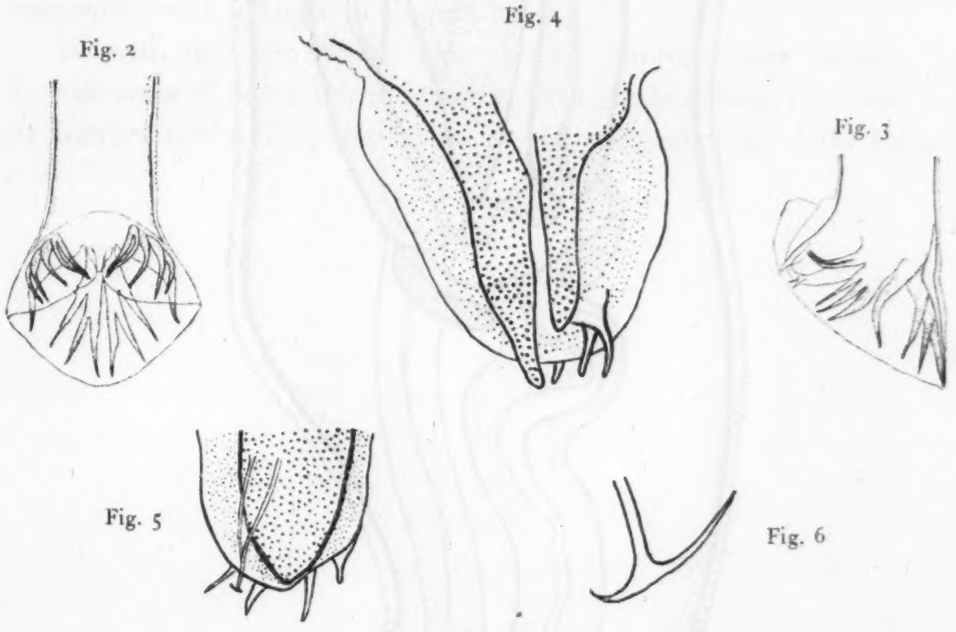
OESOPHAGUS. The length in ten males varied from 277μ to 318μ, average 298μ, and the breadth from 48μ to 63μ, average 55μ; the

ratio of breadth to length is 1 to 5·4. In ten females the length ranged from 282μ to 341μ, average 310μ, and the breadth from 43μ to 60μ, average 55μ; the ratio of breadth to length is 1 to 5·6. The ratio of the length of the oesophagus to that of the worm is in the male 1 to 14, and in the female 1 to 16.

EXCRETORY BLADDER. Lies close behind the nerve ring. The distance of its posterior margin from the posterior extremity of the oesophagus varied in ten worms from 55μ to 104μ, average 76μ.

CERVICAL PAPILLAE. Lie at about the same level as the excretory bladder.

POSTERIOR EXTREMITY OF MALE. The dorsal lobe of the bursa is relatively broad and short, if anything it is rather less than a semicircle (fig. 2). In ten worms the length of the main trunks of the



FIGS. 2-6. *Cylicostomum minutum* sp. n.

Fig. 2: Posterior extremity of male, ventral view, × 90. Fig. 3: Posterior extremity of male, lateral view, × 90. Fig. 4: Genital cone and appendages, lateral view, × 360. Fig. 5: Genital appendages, ventral view, × 360. Fig. 6: End of spicule, × 1360.

posterior rays, from the tip to the point of origin of the postero-external ray, varied from 140μ to 198μ, average 164μ. The ratio of the average length of the main trunk of the posterior ray to the average length of the male worm is 1 to 26.

Genital cone. The dermal collar is extremely well developed, both on the dorsal and ventral surfaces of the genital cone. The genital appendages are slightly elevated bodies having on their posterior surfaces two long finger-like processes; they do not meet in the middle line (figs. 4 and 5).

Spicules. The ends of the spicules are barbed, as shown in fig. 6.

POSTERIOR EXTREMITY OF FEMALE. The end of the body is sometimes almost straight, but more usually exhibits a slight S-shaped bend, turning first slightly ventrally and then slightly dorsally (fig. 7). The body tapers to the tail. The distance between the

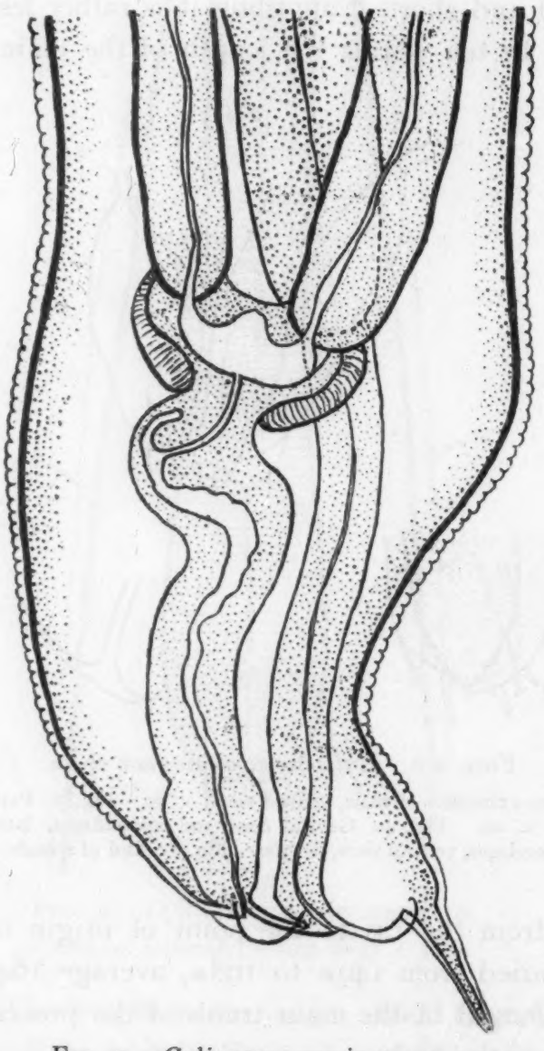


FIG. 7. *Cylicostomum minutum* sp. n.
Posterior extremity of female, lateral view, × 360.

anus and vulva varied from 21 μ to 101 μ, average 44 μ. The distance measured straight along the middle from the tip of the tail to a line drawn horizontally through the anus varied from 35 μ to 70 μ, average 50 μ.

DIAGNOSIS. The following are the chief diagnostic characters of this species:—

1. Very small size; average length, male 4·3 mm. and female 4·9 mm.

2. Buccal capsule: anterior opening circular; walls, when seen in optical section, straight; ratio of breadth at anterior opening to antero-posterior diameter 1 to 1·4.

3. Dorsal oesophageal gutter projects far into buccal capsule.

4. Dorsal lobe of bursa short, less than a semicircle; ratio of length of posterior ray to total length of male worm 1 to 26.

5. Termination of female body sometimes straight, but more frequently with a slight S-shaped bend.

It will be seen that this species approximates closely to *C. calicatum* (Looss), but is at once distinguished from the latter by its smaller size and by the shortness of the dorsal lobe of the bursa.

STRONGYLIDAE IN HORSES

III. *CYLICOSTOMUM NASSATUM*, Looss. var. *PARVUM*

BY

WARRINGTON YORKE

AND

J. W. S. MACFIE

(*Received for publication 7 February*, 1918)

SIZE AND SHAPE. A moderately small species of the GENUS *Cylicostomum*, the female being distinctly larger than the male. Ten males and ten females were measured. The males were from 6·5 mm. to 7·5 mm. in length, average 7 mm., the females were from 8·2 mm. to 9·7 mm. in length, average 8·8 mm.; the greatest breadth, in those worms which were properly orientated (lying on dorsal surface), averaged, males 347μ, females 426μ.

HEAD. The neck separating the head from the body is barely perceptible.

Mouth collar. Marked off from the rest of the skin by a deep constriction. The mouth is ellipsoidal in transverse section, the lateral diameter being greater than the dorso-ventral.

Head papillae. Submedian project anteriorly beyond the external leaf crown, their extremities are not separated off by lateral notches; lateral, very prominent, forming small ear-like projections.

Mouth capsule. Ellipsoidal in transverse section, the ratio of the lateral diameter to the dorso-ventral diameter of the anterior opening of the buccal capsule being about 1·5 to 1. When the worm is properly orientated the walls of the mouth capsule seen in optical section are straight, slender and parallel, with a well marked hoop-like thickening posteriorly (fig. 1). When viewed laterally the walls diverge slightly from before backwards, and the ventral wall is distinctly longer than the dorsal owing to the floor of the buccal

capsule being set obliquely, so that the cavity is considerably deeper ventrally than dorsally. In the males the antero-posterior diameter (i.e. the distance from the anterior to the posterior opening) of the buccal capsule varies from 22μ to 28μ, average 26μ; in the females from 28·5μ to 35μ, average 32μ.

In the males the lateral diameter of the buccal capsule, which is the same at the anterior and posterior openings, varies from 68μ to 79μ, average 71μ, and in the females from 88μ to 114μ, average 104μ. The ratio of the lateral diameter of the anterior opening of the buccal capsule to the antero-posterior diameter is, in the males 2·7 to 1, and in the females 3·2 to 1.

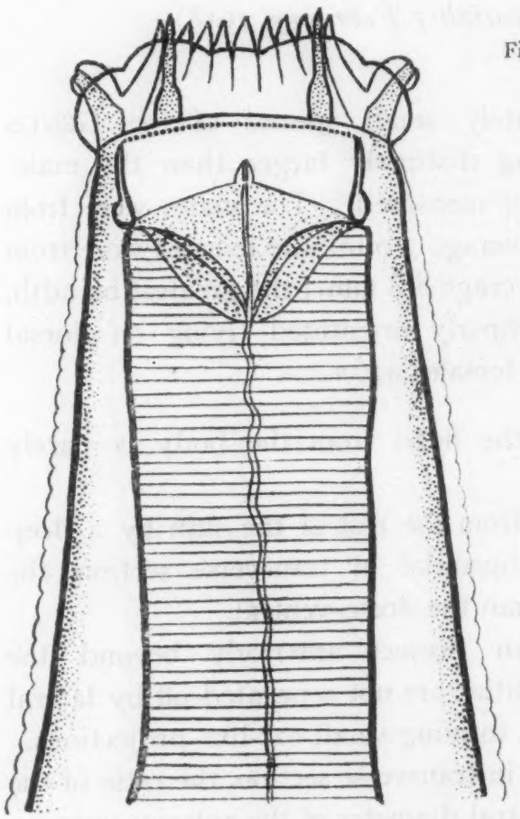


Fig. 1

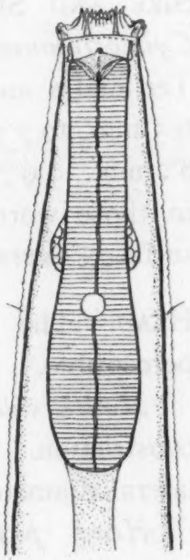


Fig. 2

FIGS. 1-2. *Cylicostomum nassatum* var. *parvum*.

Anterior extremity, ventral view; fig. 1 × 360, fig. 2 × 90.

Dorsal oesophageal gutter. Projects into the buccal capsule, reaching nearly half-way to the anterior orifice.

Leaf crowns. The external leaf crown consists of twenty large pointed elements arising from the mouth collar. The internal leaf

crown consists of a large number of minute rectangular plates arising from the anterior margin of the buccal capsule.

OESOPHAGUS. The length in ten males varied from 492μ to 603μ, average 544μ, and the breadth from 110μ to 134μ, average 120μ; the ratio of breadth to length is 1 to 4·5. In ten females the length ranged from 572μ to 649μ, average 611μ, and the breadth from 122μ to 175μ, average 148μ; the ratio of breadth to length is 1 to 4·1. The ratio of the length of the oesophagus to that of the worm is, in the male 1 to 13, and in the female 1 to 14.

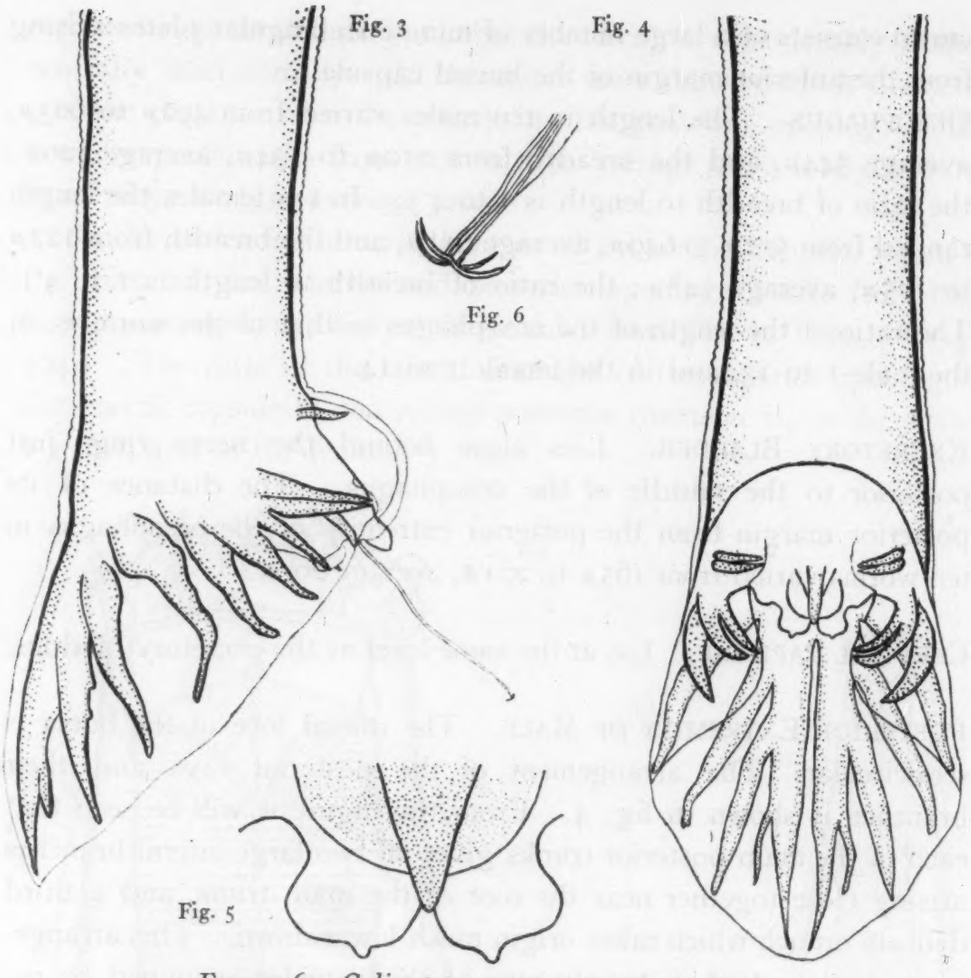
EXCRETORY BLADDER. Lies close behind the nerve ring, just posterior to the middle of the oesophagus. The distance of its posterior margin from the posterior extremity of the oesophagus in ten worms varied from 165μ to 271μ, average 207μ.

CERVICAL PAPILLAE. Lie at the same level as the excretory bladder.

POSTERIOR EXTREMITY OF MALE. The dorsal lobe of the bursa is semicircular. The arrangement of the posterior rays and their branches is shown in fig. 4. From this figure it will be seen that each of the main posterior trunks gives off two large lateral branches arising close together near the root of the main trunk, and a third delicate branch which takes origin much lower down. This arrangement was constant in twenty-nine of thirty males examined by us. In the remaining one the arrangement held on one side, but on the other side the branches were irregular. In ten worms the length of the main trunks of the posterior ray, from the tip to the point of origin of the postero-external rays, varied from 312μ to 400μ, average 363μ. The ratio of the average length of the main trunk of the posterior ray to the average length of the male is 1 to 19.

Genital cone. The dermal collar is well developed on both the dorsal and ventral surfaces of the genital cone. The genital appendages when seen from the ventral surface are broad and united in the middle line, and each bears a single papillary process; when viewed laterally they are bluntly conical, bearing on their extremities the small papillary process (figs. 3 and 5).

Spicules. The ends of the spicules are barbed in an anchor-like manner (fig. 6).



FIGS. 3-6. *Cylicostomum nassatum* var. *parvum*.

Fig. 3: Posterior extremity of male, lateral view, × 90. Fig. 4: Posterior extremity of male, ventral view, × 90. Fig. 5: Genital appendages, ventral view, × 360. Fig. 6: Ends of the spicules, × 1360.

POSTERIOR EXTREMITY OF FEMALE. The end of the body is slightly bent dorsally, and tapers gradually. The tail which is distinctly demarcated from the end of the body is straight, and tapers to a point (fig. 7). The distance between the anus and vulva varied from 98μ to 126μ, average 112μ; and the distance measured straight along the middle of the tail from the tip to a line drawn horizontally through the anus varied from 165μ to 210μ, average 184μ.

DIAGNOSIS. The following are the chief diagnostic characters of this worm:—

1. Small size: average length, males 7μ and females 8·8μ.
2. Buccal capsule: anterior opening ellipsoidal, ratio of lateral

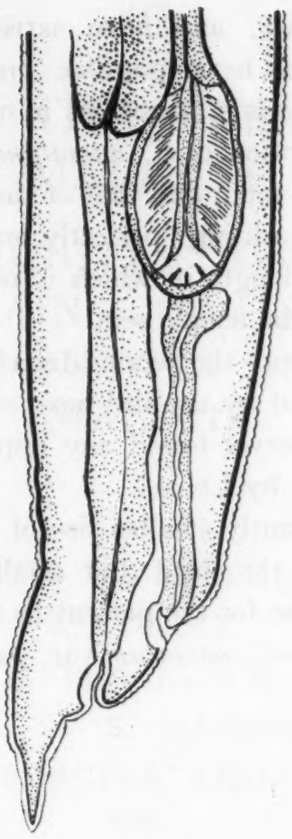


FIG. 7. *Cylicostomum nassatum* var. *parvum*.

Posterior extremity of female, lateral view, × 90.

to antero-posterior diameter of anterior opening of capsule is 1·5 to 1; walls when seen in optical section straight and slender, with a well marked hoop-like thickening posteriorly; ratio of breadth at anterior opening to antero-posterior diameter 2·7 to 1.

3. Dorsal oesophageal gutter projects well into buccal capsule.

4. Dorsal lobe of bursa moderately long; ratio of length of posterior ray to total length of male worm 1 to 19.

5. Termination of female body slightly bent dorsally.

Apart from its small size and from the possession of a third (small) lateral branch of the posterior ray, this worm is indistinguishable from *C. nassatum* (Looss). It will be noted that Looss (1901) does not indicate the prominent character of the lateral head papillae in his drawing of *C. nassatum*, but as we are fortunate enough to possess co-types kindly sent to this laboratory by Looss some years ago, we have had an opportunity of comparing our worm with the

co-types of *C. nassatum*, and have satisfied ourselves that in *C. nassatum* the lateral head papillae project as small ear-like processes in exactly the same manner as in our worm.

Looss, in his description of *C. nassatum*, states that the length of the males is about 10 mm., and that of the females up to 14 mm., but he qualifies this by adding 'perfectly mature individuals being, however, met with, the length of which is not more than 8 mm. in the male and 9 mm. in the female sex.'

So far we have found the worm described above in three of fourteen horses examined by us, and have examined large numbers of specimens, but have never found any approaching the usual size of *C. nassatum* as given by Looss.

In view of the constantly smaller size of the worm found by us, and of the existence of the third (but small) lateral branch of the posterior ray, we propose for the present to consider the worm as a variety of *C. nassatum*—*C. nassatum* var. *parvum*.

REFERENCE

Looss, A. (1901). The Sclerostomidae of Horses and Donkeys in Egypt. *Records of the School of Medicine*, Cairo, Vol. I.

STUDIES IN THE TREATMENT OF MALARIA

X. ORAL ADMINISTRATION OF QUININE SULPHATE GRAINS 120 ON TWO CONSECUTIVE DAYS ONLY, IN SIMPLE TERTIAN MALARIA

BY

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Undertaken at the request of the War Office

(Received for publication 5 December, 1917)

In No. VI (1918) of these Studies we recorded the results of the oral administration of quinine sulphate in doses varying from grains 5 to grains 90 daily on each of two consecutive days. As it seemed likely that in the Grains 90 series we were approaching the toxic dose of quinine sulphate we proceeded to ascertain whether still larger doses could be tolerated, and if so what would be the therapeutic result. With this object in view a series of cases was treated with grains 120 on each of two consecutive days. Quinine sulphate in solution was given every four hours during a period of forty-eight hours (eight 15-grain doses and four 30-grain doses). All the cases were adult males infected in Macedonia at least nine months previously, and all had had more or less quinine during this

Summary of results of oral administration of Quinine sulphate in solution, grains 120, on each of two consecutive days in simple tertian malaria.

Number of case	Temperature fell to normal in — days after first dose	Parasites disappeared from cutaneous blood in — days after first dose	Parasitic relapse occurred in — days after first dose	Febrile relapse (above 100° F.) occurred in — days after first dose	Observation period in cases which did not relapse	Remarks
692	3	Same day	...	...	66 days	100° F. on 15th day
693	Same day	1	...	...	79 days	
694	Same day	1	15	18	...	
695	1	1	...	...	74 days	
696	Apyrexia	2	12	17	...	
697	Apyrexia	1	12	19	...	
698	1	2	18-19	19	...	
699	3	1	24	26	...	
700	1	2	...	...	62 days	
701	4	Same day	18	14	...	
702	1	Same day	13	18	...	1st day grains 105; 2nd day none
703	Same day	1	...	...	60 days	1st day grains 105; 2nd day none
704	Same day	Same day	14	15	...	1st day grains 105; 2nd day none
705	1	2	...	...	72 days	1st day grains 90; 2nd day grains 105
706	1	1	14	15	...	1st day grains 120; 2nd day grains 60

period. In five of the fifteen cases (Nos. 702-706) it was found impossible to complete the treatment, owing to the development of serious symptoms: vomiting, deafness, dimness of vision, temporary blindness, collapse; but in no case did the symptoms persist beyond a week. As the majority of the other cases exhibited these symptoms in a less degree we conclude that for the average case a dose of grains 120 on each of two consecutive days is the maximum amount that can be tolerated.

The results are recorded in the Table. Parasites disappeared from the cutaneous blood either on the day of the first dose or within one to two days. In thirteen cases the temperature fell to normal either on the day of the first dose or within one to four days, whilst in the remaining two treatment was given during an apyrexial period.

Relapses. Blood examinations were made daily in all cases. Parasitic relapses occurred in twelve to twenty-four days in six of the ten cases who received the full treatment. In the remaining four cases there was no parasitic relapse within an observation period of sixty-two to seventy-nine days.

Febrile relapses occurred in fourteen to twenty-six days in six of the ten cases. In the remaining four cases there was no febrile relapse within an observation period of sixty-two to seventy-nine days.

CONCLUSIONS

Quinine sulphate orally in doses of grains 120 on each of two consecutive days represents the maximum amount of the drug which can be tolerated by the average case, as the treatment had to be abandoned owing to severe symptoms in five out of fifteen cases.

Relapses occurred in 60 per cent. of the cases who completed the treatment. The results accordingly compare unfavourably with those obtained in the Grains 90 series, where only 38 per cent. relapsed; but in the present series the number of cases treated (ten) is too small to have a real comparative value.

REFERENCE

STEPHENS, J. W. W., YORKE, W., BLACKLOCK, B., MACFIE, J. W. S., and COOPER, C. F. (1918). *Ann. Trop. Med. & Parasitol.*, Vol. XI, pp. 283-307.

Summary of results of oral administration of Quinine sulphate in solution, grains 120, on each of two consecutive days in simple tertian malaria.

Number of case	Temperature fell to normal in — days after first dose	Parasites disappeared from cutaneous blood in — days after first dose	Parasitic relapse occurred in — days after first dose	Febrile relapse (above 100° F.) occurred in — days after first dose	Observation period in cases which did not relapse	Remarks
692	3	Same day	...	...	66 days	100° F. on 15th day
693	Same day	1	...	...	79 days	
694	Same day	1	15	18	...	
695	1	1	...	...	74 days	
696	Apyrexia	2	12	17	...	
697	Apyrexia	1	12	19	...	
698	1	2	18-19	19	...	
699	3	1	24	26	...	
700	1	2	...	...	62 days	
701	4	Same day	18	14	...	
702	1	Same day	13	18	...	1st day grains 105; 2nd day none
703	Same day	1	...	...	60 days	1st day grains 105; 2nd day none
704	Same day	Same day	14	15	...	1st day grains 105; 2nd day none
705	1	2	...	...	72 days	1st day grains 90; 2nd day grains 105
706	1	1	14	15	...	1st day grains 120; 2nd day grains 60

period. In five of the fifteen cases (Nos. 702-706) it was found impossible to complete the treatment, owing to the development of serious symptoms: vomiting, deafness, dimness of vision, temporary blindness, collapse; but in no case did the symptoms persist beyond a week. As the majority of the other cases exhibited these symptoms in a less degree we conclude that for the average case a dose of grains 120 on each of two consecutive days is the maximum amount that can be tolerated.

The results are recorded in the Table. Parasites disappeared from the cutaneous blood either on the day of the first dose or within one to two days. In thirteen cases the temperature fell to normal either on the day of the first dose or within one to four days, whilst in the remaining two treatment was given during an apyrexial period.

Relapses. Blood examinations were made daily in all cases. Parasitic relapses occurred in twelve to twenty-four days in six of the ten cases who received the full treatment. In the remaining four cases there was no parasitic relapse within an observation period of sixty-two to seventy-nine days.

Febrile relapses occurred in fourteen to twenty-six days in six of the ten cases. In the remaining four cases there was no febrile relapse within an observation period of sixty-two to seventy-nine days.

CONCLUSIONS

Quinine sulphate orally in doses of grains 120 on each of two consecutive days represents the maximum amount of the drug which can be tolerated by the average case, as the treatment had to be abandoned owing to severe symptoms in five out of fifteen cases.

Relapses occurred in 60 per cent. of the cases who completed the treatment. The results accordingly compare unfavourably with those obtained in the Grains 90 series, where only 38 per cent. relapsed; but in the present series the number of cases treated (ten) is too small to have a real comparative value.

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STEPHENS, J. W. W., YORKE, W., BLACKLOCK, B., MACFIE, J. W. S., and COOPER, C. F. (1918). *Ann. Trop. Med. & Parasitol.*, Vol. XI, pp. 283-307.

STUDIES IN THE TREATMENT OF MALARIA

XI. ORAL ADMINISTRATION OF QUININE SULPHATE GRAINS 90 ON TWO CONSECUTIVE DAYS WEEKLY OVER A PERIOD OF THREE WEEKS, IN SIMPLE TERTIAN MALARIA

BY

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Undertaken at the request of the War Office

(*Received for publication* 10 *December*, 1917)

In No. VI (1918) of these studies we recorded that as the result of the oral administration of grains 90 of quinine sulphate on two consecutive days only, 62 per cent. of cases were cured, i.e. they did not relapse within an observation period varying from sixty to one hundred and sixty-five days. We then proceeded to ascertain whether a still better therapeutic result could be obtained by giving the same dose on two consecutive days each week for a period of three weeks. All the cases were adult males infected in Macedonia at least nine months previously, and all had had more or less quinine during this period. Quinine sulphate in solution was given in 30-grain doses three times on each of the two consecutive days in twenty-four cases. The results are recorded in the Table.

Summary of results of oral administration of quinine sulphate in solution, grains 90, on each of two consecutive days each week for a period of three weeks in simple tertian malaria.

Number of case	Temperature fell to normal in — days after first dose	Parasites disappeared from cutaneous blood in — days after first dose	Parasitic relapse occurred in — days after last dose	Febrile relapse (above 100° F.) occurred in — days after last dose	Observation period in cases which did not relapse	Remarks
707	1	1	...	...	108 days	
708	1	3	...	...	67 days	
709	1	2	...	...	103 days	100·8° F. on 50th day
710	1	2-3	...	...	63 days	101° F. on 40th day
711	Same day	1	21	19	...	
712	Same day	1	...	...	60 days	
713	1	1	...	...	86 days	
714	1	1	31	31	...	
715	1	1	...	...	32 days	
716	1	2	31	33	...	Blackwater after 1st dose
717	2	3	14	13	...	
718	Same day	1	12	11	...	
719	Same day	2	13	15	...	
720	Same day	1	...	...	92 days	
721	Same day	Blood* negative	57	...	...	Quinine orally on 62nd day
722	1	1	37	39	...	
723	Apyrexia	1	3	4	...	
724	1	1	15	...	...	Quinine orally on 17th day
725	1	2	...	...	60 days	
726	2	1-2	...	...	60 days	
727	Apyrexia	1	...	...	63 days	
728	1	1	20	21	...	
729	1	1	...	...	91 days†	100·4° F. on 11th day; 100·6° F. on 14th day
730	1	1	...	...	66 days††	

* Parasites 2 days previously.

† This case relapsed on the 127th day.

†† This case relapsed on the 103rd day.

Parasites disappeared from the cutaneous blood in one to three days. In twenty-two cases the temperature fell to normal either on the day of the first dose or within one to two days, while in the remaining two cases treatment was given during an apyrexial period.

Relapses. Blood examinations were made daily in all the cases. In eleven of the twenty-four cases a parasitic relapse occurred in three to fifty-seven days; in twelve cases there was no parasitic relapse within an observation period of sixty to one hundred and eight days; in one case (No. 715) the observation period was only thirty-two days. In nine cases febrile relapses occurred in four to thirty-nine days; in two cases (Nos. 721 and 724) the patient was given quinine orally a few days after the parasitic relapse.

TOLERANCE OF TREATMENT

In some of the cases deafness was marked and persisted for several days, while others complained of dimness of vision which disappeared in a few days; buzzing in the ears, giddiness, vomiting and tremors were fairly pronounced. In no case did any ill-effect persist for more than a few days.

One case (No. 716) had an attack of blackwater fever during the first treatment. Symptoms disappeared in two days. The second and third treatments were not followed by haemoglobinuria.

CONCLUSION

Quinine sulphate grains 90 on two consecutive days weekly over a period of three weeks cures* 50 to 54 per cent. of cases. These figures are therefore not quite so good as those obtained in the series treated with grains 90 on two consecutive days only, but the slight difference in the result may well be due to the smaller number of cases treated.

REFERENCE

STEPHENS, J. W. W., YORKE, W., BLACKLOCK, B., MACFIE, J. W. S., and COOPER, C. F. (1918). *Ann. Trop. Med. & Parasitol.*, Vol. XI, pp. 283-307.

* By 'cure' we mean no relapse during an observation period of sixty days after cessation of treatment. The smaller figure (50 per cent.) is calculated on the assumption that case 715, which was observed for 32 days only, relapsed before the sixtieth day.

STUDIES IN THE TREATMENT OF MALARIA

XII. AT WHAT TIME AFTER CESSATION OF QUININE TREATMENT DO RELAPSES OCCUR IN SIMPLE TERTIAN MALARIA?

BY

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Undertaken at the request of the War Office

(*Received for publication* 12 *February*, 1918)

The primary object of this analysis is to determine the shortest period after cessation of treatment for which it is necessary to keep under observation a case of simple tertian malaria, so that the probability of a relapse occurring after discharge from hospital is reasonably small. With this in view, we have analysed the time incidence of relapses in four hundred and five cases treated by us in various ways as recorded in previous papers. Almost all these cases were adult males infected in Macedonia six to twelve months previously, and all had had more or less quinine during that period.

We consider parasitic relapses only, as we know nothing of the nature of febrile paroxysms unassociated with parasites in the peripheral blood.

The data on which this paper is based are given in Tables I to III. Tables I and II contain the results of those treatments where quinine was administered either on a single occasion or on two

TABLE

Relapse time after administration of quinine on either one or two consecutive days only

Observation period after cessation of treatment in 3-day periods	Treatment							
	Quinine bihydrochloride grains **5** *orally* on each of two consecutive days Cases treated: 17 Relapses: 17		Quinine sulphate grains **5** *orally* on each of two consecutive days Cases treated: 8 Relapses: 7		Quinine sulphate grains **10** *orally* on each of two consecutive days Cases treated: 10 Relapses: 10		Quinine sulphate grains **15** *orally* on each of two consecutive days Cases treated: 14 Relapses: 14	
	Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* each 3-day period	Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* each 3-day period	Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* each 3-day period	Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* each 3-day period
1-3	17	1	8	0	10	0	14	0
4-6	16	1	8	3	10	0	14	0
7-9	15	1	5	1	10	0	14	4
10-12	14	8	4	2	10	6	10	5
13-15	6	6	2	0	4	2	5	3
16-18	0	0	2	1	2	2	2	1
19-21	...	...	1	0	0	0	1	0
22-24	...	...	1	0	...	...	1	1
25-27	...	...	1	0	...	...	0	0
28-30	...	...	1	0	...	...	...	...
31-33	...	...	1	0	...	...	...	...
34-36	...	...	1	0	...	...	...	...
37-39	...	...	1	0	...	...	...	...
40-42	...	...	1	0	...	...	...	...
43-45	...	...	1	0	...	...	...	...
46-48	...	...	1	0	...	...	...	...
49-51	...	...	1	0	...	...	...	...
52-54	...	...	1	0	...	...	...	...
55-57	...	...	1	0	...	...	...	...
58-60	...	...	1	0	...	...	...	...
61-63	...	...	0	0	...	...	...	...

in those series where no curative effect was obtained.

1—7

Quinine sulphate grains **30** *orally* on each of two consecutive days Cases treated : 14 Relapses : 14		Quinine bihydrochloride grains **15** *intramuscularly* on each of two consecutive days Cases treated : 20 Relapses : 19		Quinine bihydrochloride grains **10-15** *intravenously* (single injection) Cases treated : 6 Relapses : 6		Summary of results of the seven treatments Total cases treated : 89 Total relapses : 87	
Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* each 3-day period	Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* each 3-day period	Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* each 3-day period	Total non-relapse cases still in hospital at *beginning* of each 3-day period	Total cases relapsing *during* each 3-day period
14	0	20	0	6	0	89	1
14	0	20	0	6	1	88	5
14	2	20	3	5	2	83	13
12	5	17	5	3	1	70	32
7	5	12	10	2	2	38	28
2	0	2	1	0	0	10	5
2	2	1	0	...	...	5	2
0	0	1	0	...	...	3	1
...	...	1	0	...	...	2	0
...	...	1	0	...	...	2	0
...	...	1	0	...	...	2	0
...	...	1	0	...	...	2	0
...	...	1	0	...	...	2	0
...	...	1	0	...	...	2	0
...	...	1	0	...	...	2	0
...	...	1	0	...	...	2	0
...	...	1	0	...	...	2	0
...	...	1	0	...	...	2	0
...	...	1	0	...	...	2	0
...	...	1	0	...	...	2	0
...	...	0	0	...	...	0	0

TABLE

Relapse time after administration of quinine on either one or two consecutive days

Observation period after cessation of treatment in 3-day periods	Treatments: Quinine sulphate grains **45** *orally* on each of two consecutive days. Cases treated: 12. Relapses: 9		Quinine sulphate grains **60** *orally* on each of two consecutive days. Cases treated: 12. Relapses: 7		Quinine sulphate grains **90** *orally* on each of two consecutive days. Cases treated: 76. Relapses: 29	
	Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* each 3-day period	Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* each 3-day period	Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* each 3-day period
1-3	12	0	12	0	76	0
4-6	12	0	12	0	76	0
7-9	12	0	12	0	76	0
10-12	12	0	12	1	76	0
13-15	12	5	11	1	76	3
16-18	7	1	10	3	73	7
19-21	6	2	7	1	66	8
22-24	4	0	6	0	58	5
25-27	4	1	6	1	53	2
28-30	3	0	5	0	51	0
31-33	3	0	5	0	51	1
34-36	3	0	5	0	50	1
37-39	3	0	5	0	49	1
40-42	3	0	5	0	48	0
43-45	3	0	5	0	48	0
46-48	3	0	5	0	48	0
49-51	3	0	5	0	48	0
52-54	0	0	5	0	48	0
55-57	...	...	5	0	46	1
58-60	...	...	5	0	44	0
61-63	...	...	5	0	42	0
64-66	...	...	0	0	39	0
67-69	...	...	...	...	28	0
70-72	...	...	...	...	25	0
73-75	...	...	...	...	23	0
76-78	...	...	...	...	17	0
79-81	...	...	...	...	13	0
82-84	...	...	...	...	9	0
85-87	...	...	...	...	8	0
88-90	...	...	...	...	7	0

II

only in those series where some curative effect was obtained.

8—12

Quinine sulphate grains **120** *orally* on each of two consecutive days Cases treated : 15 Relapses : 9		Quinine alkaloid grains **15·60** *intravenously* (either one or two injections) Cases treated : 38 Relapses : 31		Summary of results of the five treatments Total cases treated : 153 Total relapses : 85	
Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* each 3-day period	Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* each 3-day period	Total non-relapse cases still in hospital at *beginning* of each 3-day period	Total cases relapsing *during* each 3-day period
15	0	38	0	153	0
15	0	38	0	153	0
15	0	38	0	153	0
15	2	38	1	153	4
13	4	37	9	149	22
9	2	28	9	127	22
7	0	19	4	105	15
7	1	15	5	90	11
6	0	10	3	79	7
6	0	7	0	72	0
6	0	7	0	72	1
6	0	7	0	71	1
6	0	7	0	70	1
5	0	7	0	69	0
5	0	7	0	69	0
6	0	7	0	69	0
6	0	7	0	69	0
6	0	7	0	66	0
6	0	7	0	64	1
6	0	7	0	62	0
5	0	0	0	52	0
4	0	...	...	43	0
3	0	...	...	31	0
3	0	...	...	28	0
2	0	...	...	25	0
1	0	...	...	18	0
1	0	...	...	14	0
0	0	...	...	9	0
...	...	...	...	8	0
...	...	...	...	7	0

TABLE

Relapse time after prolonged administration of quinine by

Observation period after cessation of treatment in 3-day periods	Treatment: Continuous									
	Quinine sulphate grains **20** *orally* daily for 14-15 weeks Cases treated : 5 Relapses : 3		Quinine sulphate grains **30** *orally* daily for 5-18 weeks Cases treated : 14 Relapses : 10		Quinine sulphate grains **30** *orally* daily for 8 weeks Cases treated : 29 Relapses : 24		Quinine sulphate grains **30** *orally* daily for 3 weeks grains **45** *orally* daily for 1 week Cases treated : 22 Relapses : 17		Quinine sulphate grains **45** *orally* daily for 3-8 weeks Cases treated : 19 Relapses : 7	
	Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* each 3-day period	Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* each 3-day period	Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* each 3-day period	Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* each 3-day period	Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* ea 3-day period
1-3	5	1	14	1	29	0	22	2	19	0
4-6	4	0	13	0	29	4	20	1	19	0
7-9	4	2	13	4	25	4	19	3	19	1
10-12	2	0	9	0	21	8	16	2	18	2
13-15	2	0	9	1	13	1	14	4	16	1
16-18	2	0	8	0	12	1	10	3	15	1
19-21	2	0	8	1	11	1	7	0	14	0
22-24	2	0	7	0	10	0	7	0	14	0
25-27	2	0	7	0	10	0	7	0	14	1
28-30	2	0	7	0	8	3	7	0	13	0
31-33	2	0	7	2	5	1	7	0	12	0
34-36	1	0	5	0	4	1	6	0	11	0
37-39	1	0	5	0	3	0	6	1	10	1
40-42	1	0	5	0	3	0	5	0	9	0
43-45	1	0	5	0	3	0	5	0	9	0
46-48	1	0	5	1	2	0	5	0	9	0
49-51	1	0	4	0	0	0	5	0	9	0
52-54	1	0	4	0	...	...	5	0	9	0
55-57	1	0	4	0	...	...	5	0	9	0
58-60	1	0	4	0	...	...	5	1	9	0
61-63	1	0	4	0	...	...	4	0	5	0
64-66	1	0	1	0	...	...	1	0	3	0
67-69	1	0	1	0	...	...	1	0	2	0
70-72	1	0	1	0	...	...	1	0	2	0
73-75	1	0	0	0	...	...	1	0	1	0
76-78	1	0	...	...	...	...	1	0	1	0
79-81	0	0	...	...	...	...	1	0	1	0
82-84	...	...	...	...	...	...	1	0	1	0
85-87	...	...	...	...	...	...	1	0	0	0
88-90	...	...	...	...	...	...	1	0	...	...

III.

either the *continuous* or the *interrupted* method.

13—21.

Interrupted

Quinine sulphate grains **10** *orally* on two consecutive days weekly for 8-16 weeks Cases treated : 17 Relapses : 7		Quinine sulphate grains **45** *orally* on two consecutive days weekly for 4-8 weeks Cases treated : 21 Relapses : 6		Quinine sulphate grains **90** *orally* on two consecutive days weekly for 3 weeks Cases treated : 24 Relapses : 11		Quinine bihydrochloride grains **10-15** *intravenously* thrice weekly for 2 weeks Cases treated : 12 Relapses : 11		Summary of results of the nine treatments Total cases treated : 163 Total relapses : 96	
Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* each 3-day period	Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* each 3-day period	Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* each 3-day period	Non-relapse cases still in hospital at *beginning* of each 3-day period	Cases relapsing *during* each 3-day period	Total non-relapse cases still in hospital at *beginning* of each 3-day period	Total cases relapsing *during* each 3-day period
17	1	21	0	24	1	12	0	163	6
16	1	21	1	23	0	12	0	157	7
15	1	20	1	23	0	12	6	150	22
13	2	19	2	23	1	6	4	127	21
11	2	16	1	22	3	2	0	105	13
9	0	15	1	19	0	2	1	92	7
8	0	14	0	19	2	1	0	84	4
8	0	13	0	17	0	1	0	79	0
8	0	13	0	17	0	1	0	79	1
8	0	13	0	17	0	1	0	76	3
7	0	13	0	17	2	1	0	71	5
6	0	13	0	14	0	1	0	61	1
6	0	13	0	14	1	1	0	59	3
6	0	13	0	13	0	1	0	56	0
4	0	13	0	13	0	1	0	54	0
2	0	13	0	13	0	1	0	51	1
1	0	13	0	13	0	1	0	47	0
1	0	13	0	13	0	1	0	47	0
0	0	13	0	13	1	1	0	46	1
...	...	13	0	12	0	1	0	44	1
...	...	9	0	9	0	1	0	33	0
...	...	7	0	7	0	1	0	21	0
...	...	2	0	6	0	1	0	14	0
...	...	2	0	5	0	1	0	13	0
...	...	1	0	5	0	1	0	10	0
...	...	1	0	5	0	0	0	9	0
...	...	1	0	5	0	...	...	8	0
...	...	1	0	5	0	...	...	8	0
...	...	1	0	5	0	...	...	7	0
...	...	1	0	4	0	...	...	6	0

consecutive days only, and are therefore comparable. In Table I are recorded those treatments in which practically no curative effect was obtained (eighty-nine cases, eighty-seven relapses), whilst in Table II are recorded those treatments in which a definite curative effect was obtained (one hundred and fifty-three cases, eighty-five relapses). In Table III are grouped together the results obtained by us with various prolonged treatments, *continuous* or *interrupted*. In most of these some curative effect was obtained (one hundred and sixty-three cases, ninety-six relapses).

One of the first questions that present themselves is whether the time incidence of relapses varies at all with the nature of the treatment employed; for example, do relapses occur more quickly after a small dose of quinine than after a large dose?

Owing to the relatively small number of cases in many of the Series of treatments 1 to 21, it is not practicable to compare the time incidence of relapses in each of the individual series. By arranging the series in groups as has been done in Tables I to III, numbers are obtained which are sufficiently large to permit of comparison. In order to decide whether the time incidence of relapses is dependent on the nature of the treatment

TABLE IV.

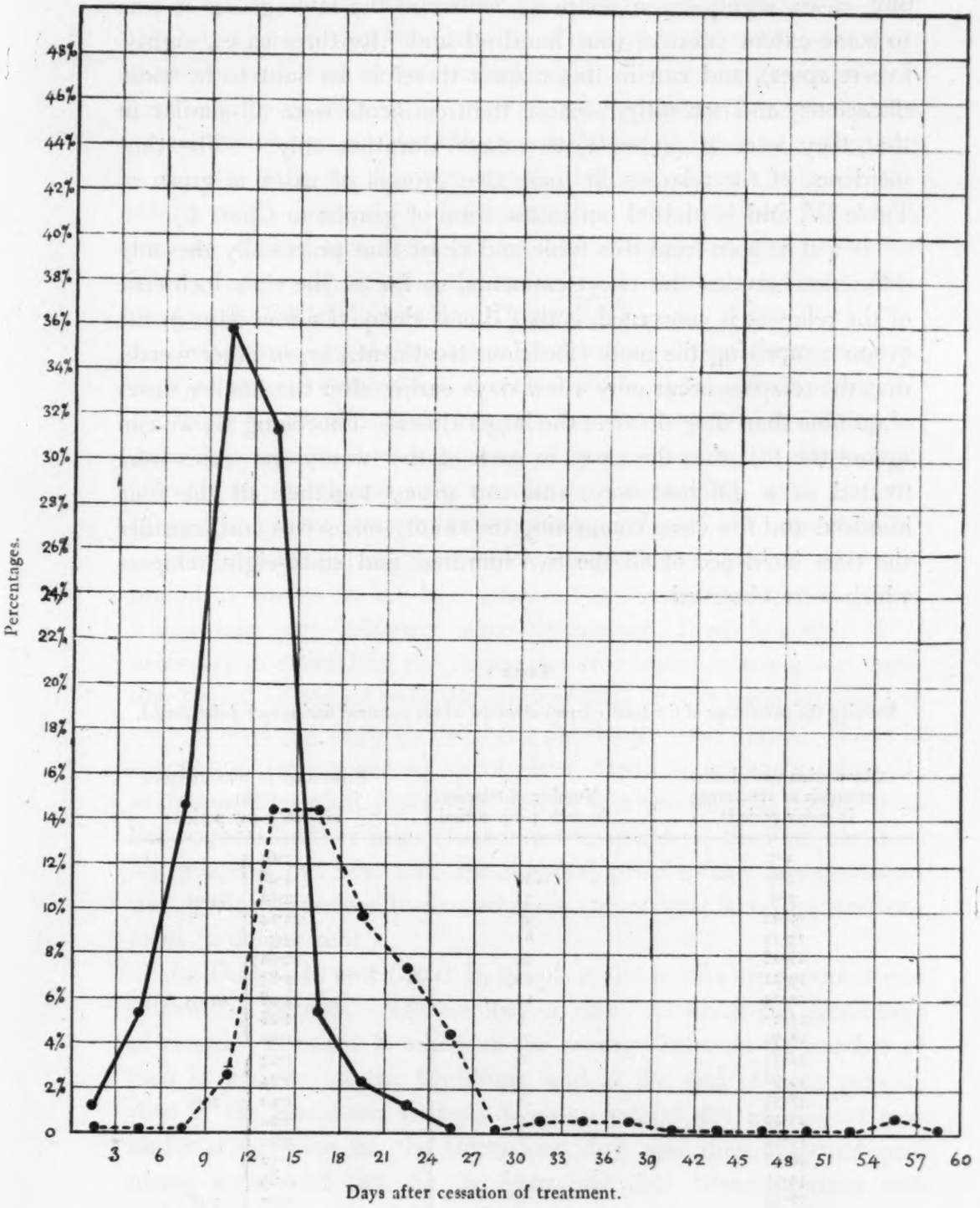
Comparing the percentage of cases treated which relapsed during each 3-day period after cessation of treatment in Series 1-7 (Table I) with those in Series 8-12 (Table II).

Observation period after cessation of treatment in 3-day periods	Number of cases treated on which the percentage of relapses is calculated		Number of cases which relapse during each 3-day period		Percentage of cases treated which relapse during each 3-day period	
	Series 1-7	Series 8-12	Series 1-7	Series 8-12	Series 1-7	Series 8-12
1-3	89	153	1	0	1·12	0·00
4-6	89	153	5	0	5·60	0·00
7-9	89	153	13	0	14·60	0·00
10-12	89	153	32	4	35·95	2·61
13-15	89	153	28	22	31·44	14·45
16-18	89	153	5	22	5·60	14·45
19-21	89	153	2	15	2·24	9·80
22-24	89	153	1	11	1·12	7·18
25-27	89	153	0	7	0·00	4·57
28-30	...	153	...	0	...	0·00
31-33	...	153	...	1	...	0·65
34-36	...	153	...	1	...	0·65
37-39	...	153	...	1	...	0·65
40-42	...	153	...	0	...	0·00
43-45	...	153	...	0	...	0·00
46-48	...	153	...	0	...	0·00
49-51	...	153	...	0	...	0·00
52-54	...	150	...	0	...	0·00
55-57	...	148	...	1	...	0·67
58-60	...	147	...	0	...	0·00

CHART I.

Percentage of cases treated which relapse during each 3-day period.

Series 1—7 ——; Series 8—12 - - - -



employed, we have selected for comparison the results obtained in Series 1 to 7 (Table I) and those in Series 8 to 12 (Table II); firstly, because treatment in the earlier series was purely palliative (eighty-nine cases, eighty-seven relapses), whilst in the later group it was to some extent curative (one hundred and fifty-three cases, eighty-five relapses), and can in this respect therefore be said to be more efficacious; and secondly, because the treatments were all similar in that they were of (at most) two days' duration only. The time incidence of the relapses in these two groups of cases is given in Table IV, and is plotted out in the form of graphs in Chart I.

It will be seen from this table and chart that practically the only difference between the two treatments, so far as the time incidence of the relapses is concerned, is that this is delayed a few days in the group comprising the more efficacious treatments, or, in other words, that the relapses occur only a few days earlier after the smaller doses of quinine than they do after the larger doses. This being so, we can ignore the fact that the cases in each of the twenty-one series were treated in a different way, and can group together all the four hundred and five cases comprising the twenty-one series and consider the time incidence of all the two hundred and sixty-eight relapses which were observed.

TABLE V.

Showing the percentage of the total relapses observed which occurred during each 3-day period.

Observation period after cessation of treatment in 3-day periods	Number of relapses in each 3-day period	Percentage of total relapses (268) in each 3-day period	
1-3	7	2·61	
4-6	12	4·48	
7-9	34	12·68	64·91
10-12	58	21·64	
13-15	63	23·50	
16-18	34	12·68	
19-21	21	7·83	
22-24	12	4·48	29·09
25-27	8	2·98	
28-30	3	1·12	
31-33	6	2·23	
34-36	2	0·74	
37-39	4	1·49	4·46
40-42	0	0·00	
43-45	0	0·00	
46-48	1	0·37	
49-51	0	0·00	
52-54	0	0·00	1·48
55-57	2	0·74	
58-60	1	0·37	
Total	268	99·94	

The time incidence of relapses can be considered in three ways:—

1. *In reference to the relapses themselves, i.e. the percentage of the total relapses which occurred during each period of time.*

Table V shows the number of relapses that occurred in each three-day period after the cessation of treatment and what percentage these numbers form of the total relapses. The percentage of relapses gradually rises from 2·61 in the one to three-day period to a maximum of 23·5 in the thirteen to fifteen-day period; after this it rapidly falls to the fifty-eighth day, subsequent to which no relapse was observed.* Grouping the incidence into fifteen-day periods, we find that 64·91 per cent. of relapses occurred in the first fifteen-day period, 29·09 in the second, 4·46 in the third, and 1·48 in the fourth (Table V and Chart II, graph 1).

2. *In reference to the total cases treated, i.e. the percentage of the total cases treated which relapse during each period of time.*

Table VI shows that the relapses increase from seven in the one to three-day period to a maximum of sixty-three in the thirteen to fifteen-day period, and subsequently decrease rapidly until, after the fifty-eight to sixty-day period, no further relapses were observed.*

The percentage of cases which relapsed during each three-day period is shown in the last column. As from time to time cases which had not relapsed were discharged from hospital, it is necessary in estimating the percentage of relapses in any given three-day period to deduct from the original total of four hundred and five such non-relapse cases as had been previously discharged. Thus in the period twenty-two to twenty-four days, twelve cases relapsed; in determining what percentage this is, it is necessary to deduct from the original total of cases (four hundred and five) the four who had not relapsed but who had left hospital prior to the twenty-second day, giving therefore twelve relapses out of four hundred and one cases (2·99 per cent.).

In Table VII and Chart II, graph 2, the results are grouped into fifteen-day periods. The number of cases on which the percentage of relapses is based is taken as the average between the number of men in hospital at the beginning and at the end of each period; thus in the thirty-one to forty-five-day period the number of men under observation on the thirty-first day was three hundred and ninety-seven and that on the forty-fifth day, three hundred and eighty-nine—average three hundred and ninety-three (Table VI).

* *Vide* addendum.

CHART II.

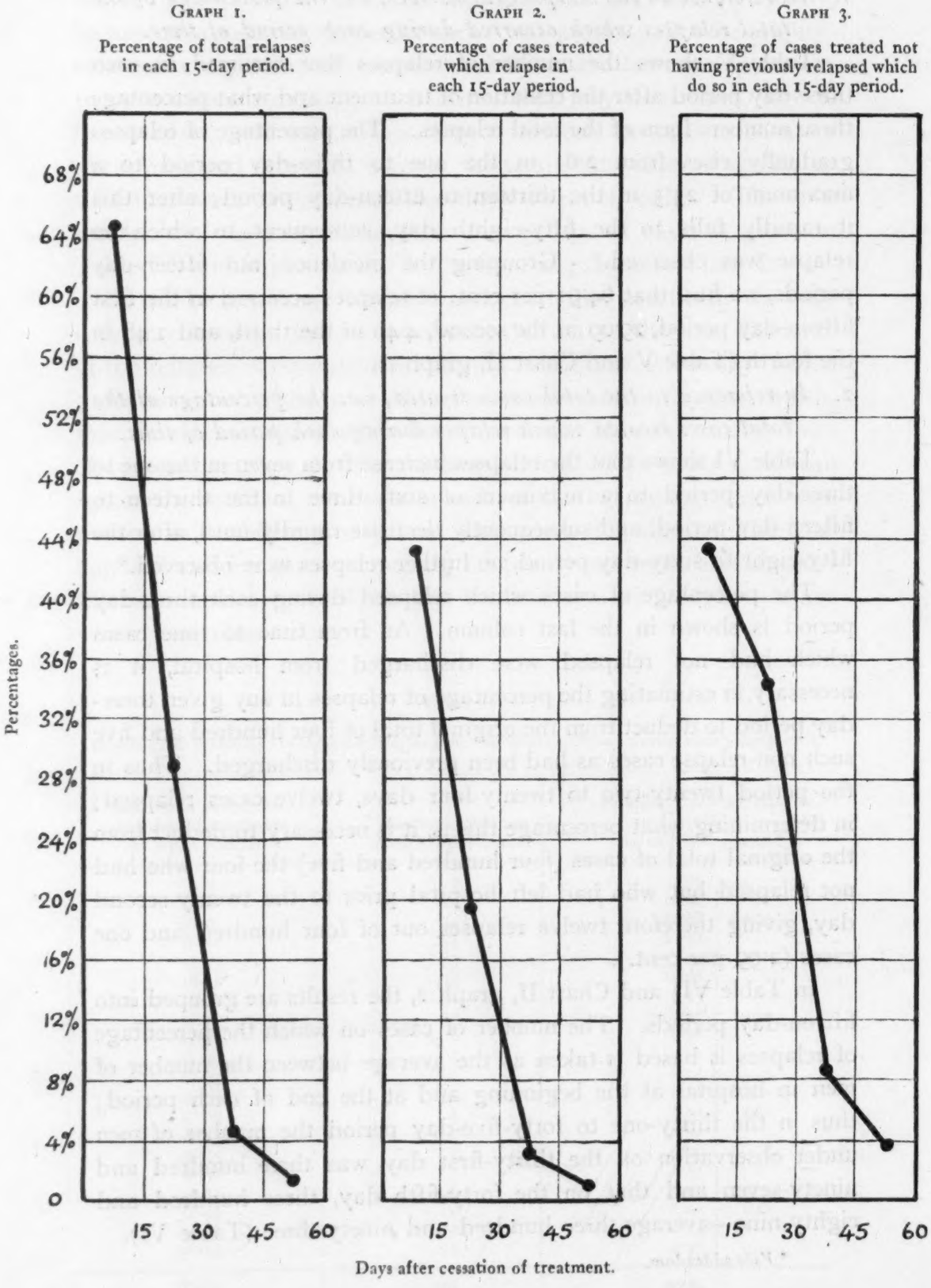

GRAPH 1.
Percentage of total relapses in each 15-day period.
GRAPH 2.
Percentage of cases treated which relapse in each 15-day period.
GRAPH 3.
Percentage of cases treated not having previously relapsed which do so in each 15-day period.
68%
64%
60%
56%
52%
48%
44%
40%
36%
32%
28%
24%
20%
16%
12%
8%
4%
0
Percentages.
15 30 45 60
15 30 45 60
15 30 45 60
Days after cessation of treatment.

TABLE VI.

Showing the percentage of cases treated which relapsed during each 3-day period after cessation of treatment.

Observation period after cessation of treatment in 3-day periods	Number of cases treated on which the percentage of relapses is calculated*	Number of non-relapse cases still in hospital at *beginning* of each 3-day period	Number of cases which relapse *during* each 3-day period	Percentage of cases treated which relapse *during* each 3-day period
1-3	405	405	7	1·73
4-6	405	398	12	2·96
7-9	405 — 404**	386	35	8·64
10-12	404	350	57	14·11
13-15	403 — [2]***	292	63	15·63
16-18	403	229	34	8·44
19-21	402	194	21	5·22
22-24	401 — 401**	172	12	2·99
25-27	401	160	8	2·00
28-30	399 — [4]	150	3	0·75
31-33	397	145	6	1·51
34-36	392	134	2	0·50
37-39	391 — 393**	131	4	1·02
40-42	391	127	0	0·00
43-45	389 — [8]	125	0	0·00
46-48	386	122	1	0·26
49-51	383	118	0	0·00
52-54	380 — 381**	115	0	0·00
55-57	377	112	2	0·53
58-60	376 — [10]	109	1	0·26
61-63	353	85	0	0·00
64-66	332	64	0	0·00
67-69	313 — 328**	45	0	0·00
70-72	309	41	0	0·00
73-75	303 — [50]	35	0	0·00
76-78	295	27	0	0·00
79-81	290	22	0	0·00
82-84	285 — 288**	17	0	0·00
85-87	283	15	0	0·00
88-90	281 — [14]	13†	0	0·00

* *i.e.*—The original number of cases (405), less those non-relapse cases who have left hospital previous to the *beginning* of each 3-day period.

** This number represents an average between two figures: (1) the total number of cases treated, less those non-relapse cases who left hospital previous to the beginning of a 15-day period; and (2) this figure less the number of non-relapse cases discharged during that period.

*** The bracketed figures represent the number of non-relapse cases discharged from hospital during the various 15-day periods.

† None of these thirteen cases relapsed in observation periods of 90, 91, 92, 93, 99, 100, 101, 102, 103, 108, 140, 164, and 165 days respectively.

The results show that of four hundred and five cases treated, approximately 43 per cent. relapse during the first fifteen days after cessation of treatment, 19·5 per cent. during the second fifteen-day period, 3 per cent. during the third, 1 per cent. during the fourth and none during the fifth and sixth fifteen-day periods or later.* These figures are not absolutely correct, because, as already explained, certain cases which had not relapsed left hospital before the observation period was complete, but as the number of these is small up to the fifty-eighth day, which was the last occasion* on which a relapse was observed, the error only affects the decimal.

TABLE VII.

Showing the percentage of cases treated which relapsed during each 15-day period after cessation of treatment.

Observation period after cessation of treatment in 15-day period	Number of cases treated on which the percentage of relapses is calculated†	Number of cases which relapsed during each 15-day period	Percentage of cases treated which relapsed during each 15-day period
1-15	404	174	43·07
16-30	401	78	19·45
31-45	393	12	3·05
46-60	381	4	1·04
61-75	328	0	0·00
76-90	288	0	0·00
91-105	276	0	0·00
106-120	271	0	0·00
121-135	271	0	0·00
136-150	270	0	0·00
151-165	270	0	0·00

* *Vide* addendum.

† *Vide* Table VI.

3. *In reference to remainders after deducting from the original total of cases treated those cases which have relapsed, i.e. the percentage of those who not having relapsed at the beginning of each period of time, do so during the period.*

From Table VIII and Chart II, graph 3, it will be seen that in the first fifteen-day period one hundred and seventy-four relapses occurred among four hundred and four cases (the average number of cases under observation during the period—four hundred and five at the beginning and four hundred and three at the end (Table VI)); this gives a percentage of relapses of 43. In the second fifteen-day period, seventy-eight relapses occurred among the remaining two hundred and twenty-seven who had not relapsed during the first fifteen-day period (two hundred and twenty-seven being the average between two hundred and twenty-nine in hospital at the beginning of the period and two hundred and twenty-five at the end, four cases having left hospital during the period (Table VI); this gives a percentage of relapses of 34·3. The percentages of relapses amongst the cases not having previously relapsed are similarly calculated for the remaining four fifteen-day periods.

TABLE VIII.

Showing what percentage of cases not having previously relapsed did so in any 15-day period.

Observation period after cessation of treatment in 15-day periods	Average number of cases in hospital which have not relapsed in each 15-day period	Number of relapses in each 15-day period	Number of relapses expressed as percentages of the non-relapse cases under observation in each 15-day period
1-15	404	174	43·0
16-30	227	78	34·3
31-45	141	12	8·5
46-60	117	4	3·4
61-75	60	0	0·00
76-90	20	0	0·00
91-105	8	0	0·00
106-120	3	0	0·00
121-135	3	0	0·00
136-150	2	0	0·00
151-165	2	0	0·00

The data as presented in Table VIII are best adapted for furnishing an answer to the problem stated at the commencement of this paper, namely, what is the shortest observation period after which a man who has not relapsed can be discharged from hospital, with but a small risk of subsequent relapse?

Table VIII shows that 43 per cent. of cases relapsed during the first fifteen days after cessation of treatment, and that 34·3 per cent. of the remainder relapsed during the next fifteen days. Only a small percentage of those who had not then relapsed did so during each of the next two fifteen-day periods, whilst in the fifth and sixth fifteen-day periods, or later, no relapses occurred. Consequently, if a man has not relapsed for fifteen days after treatment, his chance of relapsing during the next fifteen days is 34 per cent.; but if he has not relapsed for thirty days, his chance of relapsing during the following fifteen days is only 8·5 per cent., whilst if he has not relapsed for forty-five days, his chance is reduced to 3·4 per cent. during the following fifteen-day period.

The number of cases observed for periods over sixty days was an average of sixty for sixty-one to seventy-five days, twenty for seventy-six to ninety days, and thirteen for periods varying from ninety-one to one hundred and sixty-five days. No relapses were observed later than the fifty-eighth day.* Moreover, as the percentage of relapses falls so rapidly from the first to the fourth fifteen-day period, we may logically infer that only a very small proportion of those cases who have not relapsed during an observation period of sixty days will do so subsequently. This inference can only be regarded as probable, because we do not know what may happen to these cases if subjected to a different set of conditions to those under which we made our observations, viz., those of hospital patients going out daily. It may be urged also that the inference is incorrect, for it is known that cases do relapse at long intervals. We may point out, however:—(1) That we know of no series of published cases *that has been observed for sixty days or longer after treatment, and in addition has been controlled by daily blood examinations*. It is quite possible, for example, that some relapses at long intervals may represent cases which after treatment show parasites without clinical symptoms. Such cases would have been eliminated by daily blood examinations subsequent

* *Vide* addendum.

to treatment, and would not thus come into the category of late relapses. We propose to deal with this question in a future study. (2) Accepting relapses at long intervals as an established fact, we have no data to show what percentage they form of any definite series of cases treated. We would add that we are fully aware of Caccini's (1902) papers on relapses, but his data as presented are to us unintelligible.

The general conclusion to be drawn from these observations appears to be that if a case of simple tertian malaria has not relapsed parasitically within four weeks of cessation of treatment he can be discharged from hospital with a risk of relapse of only about 13 per cent., whilst if he has not relapsed within six weeks of cessation of treatment his risk of subsequent relapse is reduced to less than 5 per cent.

It must again be emphasised that the conclusions are based on the fact that frequent (in most cases daily) blood examinations were made, and consequently when we refer to a case as not having relapsed for six weeks, we mean that frequent (almost daily) examinations of his blood during that period were negative.

REFERENCE

CACCINI, A. (1902). *Journ. Trop. Med. & Hyg.*, Vol. V, p. 119 *et seq.*

ADDENDUM (13.4.18)

Since writing this paper, two of the cases considered who had not relapsed in sixty-six and ninety-one days respectively, did so on the one hundred and third and one hundred and twenty-seventh days.

DETECTION AND ESTIMATION OF QUININE IN BLOOD AND URINE

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Report to the Medical Research Committee

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Chemical investigations in connection with the treatment of malaria demand *inter alia* delicate and speedy methods for the detection and estimation of quinine in animal material. Since, at the request of the Medical Research Committee, we took up the subject we have found it possible to improve on some of the methods hitherto in use, and the observations recorded below are published at once in the hope that they will be useful to other workers. In the early part of the work we had the advantage of the co-operation of Dr. A. L. Robinson, Surgeon, R.N. (retired), but other calls on his time unfortunately made it impossible for him to continue his assistance.

QUALITATIVE TESTS

Thalleioquin. The formation of the green pigment thus named when quinine in dilute acid solution is treated with bromine water and then alkalised with ammonia, although a useful test has not proved to be a very delicate one. Giemsa and Schaumann state the dilution limit as 1 in 7,500. We find that (1) the amount of bromine water must be carefully proportioned to that of quinine—neither too little nor too much; (2) an appreciable interval must be allowed before ammonia is added; (3) the addition of ammonia must

not be delayed too long. By the following procedure we can detect with ease and certainty 0·25 mgms. of quinine in 10 c.c. of solution (= 1 in 40,000), and with difficulty 0·025 mgms. (= 1 in 400,000).

1. Pour into each of 6 small test-tubes 1 drop of strong ammonia solution.
2. To 10 c.c. of the quinine solution feebly acidified with hydrochloric acid, kept agitated and held against a white background, add 1/10th saturated bromine water drop by drop until its slight yellow colour is no longer instantly discharged. Then at intervals of 5 seconds remove successive lots of about 2 c.c. of the mixture and transfer each at once to one of the ammoniated test-tubes. Finally mix all together and extract any green pigment with amyl alcohol or chloroform.

The reaction is given also by quinicine, quinidine, di-quinicine, hydroquinine, hydroquinidine, cupreine and quitenine. It is not given by quinamine, cinchonine, cinchonicine or cinchonidine.

Fluorescence is, as already well known, detected with more certainty if a beam of strong sidelight is brought to a focus in the quinine solution with a condensing lens and inspection is made from above. The alkaloid should be dissolved in dilute sulphuric, acetic or tartaric acid, and the test-tubes should preferably be made of transparent silica. As chlorides and numerous other substances, even in traces, greatly diminish or abolish the fluorescence, the test is not a very useful one.

0·1 mgm. of quinine is detected in 4 c.c. of solution easily.

HERAPATH TEST

Owing to the low solubility of herapathite ('artificial tourmaline') in 70 per cent. alcohol, and the ease with which it forms crystals with highly characteristic polarising effects, we have found it possible to obtain them from very small amounts of quinine and to identify them in microscopic amounts by rotating a Nicol's prism above the eyepiece of the microscope.

By the following procedure we can detect with ease and certainty as little as 1/500th of a milligram of quinine in 40 c.c. of water (= 1 in 20,000,000).

1. Dissolve 5 grams of ammonium sulphate in every 10 c.c. of the quinine solution, alkalise it with ammonia and extract the quinine by shaking with 3 successive 5 c.c. lots of purified ether, transferring each lot as it separates into a small silica crucible on a water bath. Aspiration of air from its interior greatly accelerates drying.

2. Dissolve the residue when quite dry in a minimum of anhydrous ether squirted repeatedly down the sides of the crucible by means of a small teat-pipette.
3. Bring the ether solution in successive drops on to a warm microscopic slide in such a way that the residue left by its evaporation is spread over a minimal area.
4. Put on a cover-slip, add just enough of Christensen's Herapathite reagent (see appendix) to fill up about one third of the enclosed space and by gentle pressure force it into contact with all the residue left by the ether —if quinine be present, blackening will usually be visible to the naked eye. Examine microscopically and observe effects of rotating a Nicol's prism over the eyepiece on the illumination of the crystals. If only dark masses are present without appreciable polarising effects, warm the slide gently and more characteristic crystals will form as it cools.
5. Wash and heat the crucible to redness before using it again for this test.

Crystals with similar optical properties are given also by quinidine, cinchonine and cinchonidine.

Tanret's Reagent (= Mayer's Reagent). Although a fairly general precipitant for alkaloids in acid solution, this reagent attains a high degree of specificity as a test for the cinchona alkaloids when applied to the residues of ether extracts of alkalised watery solutions of animal origin, since very few other alkaloids are extracted by ether under such conditions, and these others are so potent that they are used as drugs only in very minute doses (e.g. the atropine alkaloids). It is essential, however, that the ether used should be free from all traces of aldehyde or acetone (*vide* appendix).

The clouding produced when Tanret's reagent is added to a 0·04 per cent. HCl solution of quinine clears up with moderate heat and reappears with cold. It is soluble also in ether, alcohol, urea and sodium chloride, and in excess of acid or of the reagent. Definite clouding is obtainable with 5 c.c. of solution in 0·04 per cent. HCl containing as little as 1 mgm. of quinine in 500 c.c. (= 1 in 500,000).

We have, however, made the test greatly more delicate, and, as will be seen later, the improvement is of considerable practical utility, by dissolving the quinine in saturated ammonium sulphate solution and then adding 1/100th of its volume of Tanret's reagent. Under these conditions we get a definite turbidity with as little as 1 mgm. of quinine in 10 litres of ammonium sulphate solution, and can detect in 2 c.c. of solution therefore as little as 1/5,000 mgm. Even a single drop (0·04 c.c.) of a solution containing 1 mgm. in 200 c.c.,

(= 1/5,000th mgm.) gives a definite opalescence on a slide, easily visible against a background of velvety soot. This turbidity, besides appearing in much more dilute solutions, is very much less readily soluble with warmth or in excess of the reagent or of acid. It is however easily soluble in ether or alcohol, and addition of acid renders the test definitely less sensitive. Between the effect of complete saturation with ammonium sulphate and 80 per cent. saturation there is no very marked difference, but with less than this the delicacy of the test is greatly diminished.

In view of the solubility of Tanret turbidity in excess of acid, especially in ordinary aqueous solution, it is with some surprise that we have recently read an abstract of the procedure followed by Teichmann for rough comparative estimations of quinine in blood, in which the quinine extracted by ether from 5 c.c. of blood diluted and made alkaline with NaOH, is dissolved in 1 c.c. of water plus 2 drops of strong HCl, and its amount roughly estimated by 'the intensity of the yellow tint' obtained on addition of 'Giemsa's' (and Schaumann's ?) reagent (*vide* appendix). As the reagent itself slowly becomes yellow on standing, and rapidly does so on addition of a little strong HCl, and as when using pure quinine we can find no proportion whatever between the yellow colour and the amount of quinine present, we are disposed to assume that Teichmann either gets impure quinine out of the blood or that his process is inadequately described, and that it is the intensity of the turbidity that has served for his rough estimations. If the latter is the case, it should be noted, however, that the addition of strong HCl greatly diminishes the delicacy of the test. Repeating his procedure, but with pure quinine, we find that it is impossible to get the faintest turbidity unless at least 0·010 milligram of quinine is present in 1 c.c. of water plus 2 drops of strong HCl whatever proportions of 'Giemsa's' reagent be added, whereas we had found that when the quinine was dissolved in 0·04 per cent. HCl, Tanret turbidity was easily obtained with as little as 0·002 milligram, and when dissolved in saturated $(NH_4)_2 SO_4$ solution with as little as 0·0002 mgm.

Wagner's Reagent gives a definite turbidity when added in excess to solution of quinine in dilute acid (e.g. 0·4 per cent. HCl) of dilution even as low as 1 in 1,500,000. But the solution is so highly coloured that we find it easier to work with Tanret's reagent.

Phosphotungstic Acid and Dragendorff's Bismuth Iodide Reagent are much less delicate precipitants for quinine than either Wagner's or Tanret's reagent.

QUININE IN URINE

In view of the great utility which a delicate method for detecting quinine would have if it could be applied directly to urine, we have made many attempts to improve on that used by Giemsa and Schaumann. Of the tests described above, only Wagner's and Tanret's reagents (or Giemsa's and Schaumann's modification of the latter) could possibly be applied directly to urine, but it was found that they frequently produced precipitates even in normal urine. It became necessary therefore to devise a method for removing the interfering substances of such urine while leaving behind all quinine. This, we found, could be accomplished, as far as Tanret's reagent was concerned, without the loss of any quinine whatsoever, by the following procedure. Very many variations and other procedures have been attempted, but none have hitherto proved so satisfactory. The same 'defaecation' process serves also to remove the substances which give troublesome emulsions when alkalised urine is shaken with ether.

Defaecation of Urine. For every 100 c.c. of urine add 5 c.c. of 25 per cent. lead acetate solution containing 2·5 per cent. acetic acid and mix thoroughly. Then add 5 c.c. of saturated ammonium sulphate and filter until a clear, lead-free filtrate is obtained. Receive a drop on filter paper moistened with a sulphide to test for lead.

Very exceptionally urines are met with which give a filtrate containing lead, notwithstanding the presence of excess of sulphates. We have not investigated what lead-solvent factors are concerned. Urines which yield such filtrates must either be tested without defaecation or by some indirect method.

For quantitative work take 100 c.c. of filtrate as representing 92·3 c.c. of the original urine.

Wagner's Reagent. Many normal urines, even when defaecated

as above, become turbid with this reagent—it is, therefore, not applicable directly to urine.

Tanret's Reagent. If the defaecated urine contains quinine in amounts exceeding 0·125 mgm. in 10 c.c., it will generally show a perceptible turbidity when Tanret's reagent is added drop by drop. But different urines show different degrees of turbidity even when of identical quinine content. A urine of which 10 c.c. contains 0·5 mgm. usually gives a marked turbidity. It is rare to meet with a urine other than that of a patient taking quinine which, after defaecation as above, gives a positive result with Tanret's reagent. Such urines do however exist. Influenced by speculations concerning the nature of the substance giving Tanret turbidity which is formed when alkalised urine has been shaken with commercial (aldehyde-containing) ether, we have found, for example, that hexamine and all urines containing it give precipitates with Tanret's reagent. Possibly the urine of men taking paraldehyde would do the same.

Giemsa and Schaumann have made extensive use of a modified Tanret's reagent. To guard against fallacy from the presence of coagulable protein, they recommend that after addition of the reagent the urine should be boiled and filtered hot—if the filtrate becomes cloudy when cooled the presence of quinine may be inferred. This procedure, as they point out, does not exclude fallacy from proteoses and peptones, since these substances behave like quinine, although according to them they are rarely or never present in malarial urine. To us, it appears that the 'defaecation' above described should be carried out in every case as a matter of routine—all the more so because Tanret turbidity often fails to reappear when a heated urine is cooled.

Owing to its lack of delicacy, this direct test is as a rule positive only in urine passed during the first twenty-four to thirty-six hours after a dose of the drug. During this period it is sometimes possible by comparison with urines containing known amounts of quinine even to arrive at a very rough notion of its amount. Various workers (*vide* Brault, Jeanselme et Dalimier, and Soulier), tempted by the rapidity and convenience of such 'estimates,' have made numerous observations of this kind, but, for reasons stated above, little weight can be attached to the results, except when very gross differences are concerned.

Tanret turbidity applied to Quinine extracted from Urine. Applied to the alkaloid extracted from urine by 'purified' ether, Tanret turbidity can be made an extremely delicate test—detecting with certainty as little as 0·002 milligram of quinine in 40 c.c. of urine (i.e. 1 in 20 millions).

The urine is first defaecated (to obviate emulsification of ether) as already described. 40 c.c. of the filtrate are saturated with Am_2SO_4 and shaken with ether in three successive lots to remove 'oily' matter, then alkalised with NH_4OH and again shaken up with three successive lots of 'purified' ether. The ether is evaporated off in a small silica crucible, the residual alkaloid is washed down and dissolved in two drops of 0·04 per cent. HCl squirted repeatedly from a small teat-pipette over the whole inner surface of the crucible. The solution is placed on a slide or in a small test-tube, and a micro-'drop' of the Tanret reagent is added at one side. As the only common alkaloids, other than those of cinchona, which are extracted by ether from alkalised urine, are the atropines, there is only slight risk of fallacy, but in case of doubt it is necessary to rely on the Herapathite test.

It is not permissible in the case of urine, when the presence of minutest traces of quinine is to be tested for, to dissolve the ether residue in saturated ammonium sulphate solution in order to make the Tanret test still more delicate, since even with normal urine a very slight turbidity is often obtained under those conditions. Whether this is due to some original constituent of the urine or to traces of a substance formed from impurities still present in the 'purified' ether is not yet certain. The turbidity is so slight as to be negligible in the nephelometric estimations described later.

If the methylated ether used for extracting the alkaloid be not specially purified beforehand, the dilute acid solution of the residue left on its evaporation will invariably give a precipitate with Tanret's reagent.

It may be mentioned here that Wagner's reagent gives a turbidity with the residue left by an ether extract of normal urine, even when purified ether has been used.

HERAPATH TEST

In 40 c.c. of the urine, defaecated as described already, 20 grams of $(NH_4)_2 SO_4$ are dissolved by shaking, the solution is extracted with three lots of ether to remove oily matters which interfere with the test, then alkalised with ammonia and extracted with three further 8 c.c. lots of ether. The residue left on evaporation of the ether is tested as described on page 445.

No difficulty is found in getting the characteristic crystals from 40 c.c. of urine containing as little as 1/40th of a milligram (= 1 in 1,600,000)—they can often be obtained when only 1/1,000 mgm. is present (= 1 in 4,000,000). The inferior delicacy, as compared with quinine from a pure watery solution (cf. 1/500th mgm.), is due to impurity extracted simultaneously from the urine.

This test being highly specific as well as delicate, should be carried out in all doubtful cases.

DELICACY OF TESTS FOR QUININE

	c.c. of solution containing 1 mgm.	c.c. of solution required	Mgm. of quinine detected
Thalleioquin ... ... (easily)	40	10	0·2500
,, ... (limit)	400	10	0·0250
Tanret added to solutions in 0·04 HCl ... ... (easily)	300	2	0·0066
,, ,, ,, ... (limit)	500	2	0·0040
Tanret with quinine in saturated solutions of ammonium sulphate ... ... (limit)	10000	2	0·0002
Tanret direct to urine ... ... (easily)	80	5	0·0625
,, ,, ... (limit)	200	5	0·0250
Fluorescence ... ... (easily)	40	4	0·1000
Herapathite from pure quinine ... ... (easily)	20000	40	0·0020
,, ,, ,, ... (limit)	40000	40	0·0010
Herapathite from quinine extracted from urine by rapid process ... ... (easily)	1600	40	0·0250
,, ,, ,, ,, (limit)	4000	40	0·0100

VOLUMETRIC ESTIMATION OF QUININE

As supplying a necessary criterion of the purity of the quinine extracted from animal material, or the purity being known, as a suitable method of estimating its amount, Gordin's titration method as practised by Schmitz has proved both useful and reliable. The pure quinine extracted from the urine, dissolved by the aid of a gentle warmth in a measuring flask in a known volume of N/20 sulphuric acid (e.g. 100 mgms. of alkaloid in 30 c.c. of acid) is after cooling precipitated by addition of excess of Wagner's reagent. Water is added up to the mark, the precipitate is filtered off, an aliquot part of the filtrate (which should be moderately brown in colour with excess of iodine) is decolorized by addition of a few drops of 10 per cent. sodium thiosulphate solution and titrated with N/20 sodium hydroxide. As indicator we have used methyl orange in preference to phenol-phthalein.

Theoretically 1 molecule of quinine should, when thus precipitated, take down with it 2 molecules of monobasic acid. Therefore 8·1 mgms. of quinine should remove 1 c.c. of N/20 acid.

According to Kippenberger, the method gives erroneous results with morphine and strychnine, the titration values being profoundly influenced by the proportion of free acid and also by the amount of potassium iodide present. In the case of quinine, we find no evidence of these influences within such variations in the relative amounts of the reagents as are at all likely to occur in practice. The slight variations in the results shown below suggest mere titration errors, and it should be noted that, since only half the filtrate was titrated, an error of 0·1 c.c. in titration would lead to an estimation error equal to 1·6 mgm. of total quinine, or if 100 mgms. of quinine had been taken it would give 1 c.c. of N/20 acid = 8·23, instead of the theoretical value of 1 c.c. N/20 acid = 8·1.

The titration method of Elvove had escaped our notice, but would probably have answered equally well. In this method the quinine alkaloid is treated with excess of 4 per cent. hydrochloric acid and heated for three hours on a water bath. All excess HCl escapes, the residual quinine hydrochloride is then dissolved in water and its chlorine estimated by Volhard's method.

Mgm. quinine taken	c.c. $N/20H_2SO_4$ (approx.) taken	c.c. Iodine solution	Mgm of quinine 1 c.c. of approx. $N/20H_2SO_4$	Ratios. Quin. : Acid. : Iodine
250	75	37·5	8·10	10 : 3 : 1½
100	30	15	8·16	10 : 3 : 1½
100	30	30	8·17	10 : 3 : 3
90	30	20	8·23	9 : 3 : 2
90	30	40	8·27	9 : 3 : 4
90	50	20	8·18	9 : 5 : 2
30	10	20	8·16	9 : 3 : 6
15	5	15	8·16	9 : 3 : 9
100	30	25	8·17	10 : 3 : 2½
100	60	15	8·40	10 : 6 : 1½
100	30	15	8·23	10 : 3 : 1½

GRAVIMETRIC ESTIMATION OF QUININE

Gaglio's method of evaporating the urine to dryness after addition of 5 grms. of burnt magnesia for every 100 c.c. urine, and then powdering and extracting the residue with chloroform was discarded because (1) the residue was so gummy in parts that it was impossible either to reduce it to fine powder or to transfer it completely from the drying vessel to the extraction vessel; (2) the large bulk of the residue made the extraction process tedious and large volumes of chloroform necessary; (3) the alkaloid extracted was always impure.

Kleine's Picric Acid Method. The quinine precipitated by picric acid is filtered off after twenty-four hours and the residue is boiled with 3 per cent. sodium hydrate, then shaken with chloroform for two hours in a shaker to extract quinine. The emulsion formed with the chloroform was so persistent and troublesome that we abandoned this method after a few trials, especially as Giemsa and Schaumann had found that the product yielded was yellow and impure.

Giemsa and Schaumann's Method. Render 200 c.c. of urine alkaline with sodium hydrate, shake up with three successive 50 c.c.

lots of ether, distil off the separated ether, dissolve the residue in chloroform, filter, evaporate off the chloroform and dry at 120° C.

We have experimented with this method on an extensive scale, using normal urine to which known quantities of quinine salts had been added. Among the difficulties encountered have been (1) the frequency with which troublesome emulsions are formed with ether; (2) the quinine extracted was never pure and it often had a urinous smell, it sometimes darkened when dried at 120° C., and there was considerable disparity between the amounts of quinine as found gravimetrically and as estimated by Gordin's method.

The emulsion trouble can be completely obviated in unboiled urine by 'defaecation' with lead acetate as described on page 447. As correction for the dilution involved and the volume occupied by the precipitate produced, take 100 c.c. of filtrate as representing 92·3 c.c. of original urine. If the observer prefers to dispense with this correction factor and get the whole of the quinine, the precipitate produced by the lead salt may be filtered off under suction through two flat filter papers on a Buchner filter with a sandwich layer of kieselguhr between them; the residue is then well washed with water and the washings added to the filtrate. Without this device of an upper filter paper to protect the kieselguhr from disturbance, washing without passage of some of the precipitate into the filtrate is impossible. The defaecation process is unfortunately useless when applied to urine which has been concentrated by boiling—the filtrate then obtained invariably forms troublesome emulsions with ether. Mariani's statement that abundant acidification of urine with H_2SO_4 before concentration prevents emulsion trouble does not correspond with our experience.

Applying Giemsa and Schaumann's method to defaecated urine, the product, although often having the correct weight, is impure, and gives incorrect titration values by Gordin's method.

Urine	Mgms. of quinine added	Mgms. of product found by weight	Mgms. of product equivalent to 1 c.c. N/20 H_2SO_4 found by titration	Mgms. of quinine found by titration, taking 1 c.c. of N/20 acid as equal to 8·2 mgms.
100 c.c.	100	100·1	9·5	82·7
100 c.c.	100	98·7	9·98	81·3

That the impurity of the quinine was not derived entirely from the urine was proved by putting pure aqueous solution of quinine sulphate through the same process—the quinine extracted showed much the same diminished power of fixing acid in the Gordin titration as had been observed in that from urine (or, alternatively, it contained some substance which was itself either acid in character or developed acidity under the influence of Wagner's reagent). It was evident that the methylated ether used was in this case responsible for the abnormality of the product, notwithstanding the fact that it left no residue on evaporation. It was found to give both aldehyde and ketone reactions, and in view of the 'reactivity' of these substances we repeated the experiment, using ether specially freed from them.

Using 'purified' ether, the Giemsa and Schaumann process now gave pure quinine. When applied to urine, however, the product still had a urinous smell and abnormal Gordin values, although it gave fair gravimetric results. Also control observations with normal urine often gave us a slight urinous residue. By adding to every 100 c.c. of the defaecated urine 15 grms. of sodium chloride and, while still acid, extracting it with three successive lots of pure ether, most of this urinous matter along with a considerable amount of 'oil' and pigment is removed, and a much purer quinine is eventually obtained. In one experiment we got from 100 c.c. of urine 220 milligrams of 'oily matter' by the preliminary extraction of the acid urine with ether. As thus modified, the Giemsa and Schaumann method yields a fairly pure product and results of sufficient accuracy for most clinical purposes. Two typical estimations are quoted.

Urine	Mgms. of quinine added	Mgms. of quinine found by weight	Mgms. found by Gordin titration	Mgms. of product equal to 1 c.c. of N/20 acid
200 c.c.	100	97·9	98·0	8·2
200 c.c.	100	98·5	95·4	8·4

It still suffers from two serious disadvantages, however:—

1. Large quantities of ether are necessary, and the ether must be carefully purified.

2. It cannot, owing to emulsion difficulties, be applied at all to urine which has been concentrated by boiling.

We have therefore sought for an entirely new method.

NEW PROCESS FOR ISOLATING QUININE FROM URINE

This is applicable either to pure urine or to urine concentrated to 1/20th its original volume by boiling. No emulsion troubles arise. The quinine obtained has a very high degree of purity. The time and labour involved and consumption of ether are all greatly reduced. When estimating the quinine of several litres of urine these advantages can hardly be over-estimated.

A known volume of the urine is 'defaecated' with lead acetate as before, and if the volume is large a known volume of the filtrate is concentrated in a shallow aluminium pan of suitable size (overhung by an inverted funnel to catch splashings) over a free flame to about 1/20th of its original volume. With such a pan it is easy to boil off 14 c.c. a minute. The pan is emptied into a measuring cylinder, and thoroughly washed several times with N/20 H_2SO_4. To the urine plus washings add for each 100 c.c. 2 c.c. of 10 per cent. sulphuric acid and one-tenth its volume of kieselguhr, and then while under thorough agitation add, for every 100 c.c. of the suspension, 40 c.c. of Wagner's iodine reagent. Filter, under suction, through a Buchner filter until the filtrate comes through clear. Test the filtrate with Wagner's reagent and, if necessary for complete precipitation, add more and refilter. The volume of reagent required depends mainly on the amount of quinine. Wash the residue and the precipitated urine four or five times with a mixture of Wagner's reagent and four times its volume of N/20 H_2SO_4. Dissolve the residue by sucking through it several times successive lots of 0·4 per cent. HCl, plus enough sodium bi-sulphite solution to decolorize it. Use about 100 c.c. altogether—the filtrate will usually be pale yellow with urinary pigment. To the filtrate add for every

10 c.c. five grams of ammonium sulphate and shake up with successive lots of ether until it ceases to extract pigment. Finally alkalise with ammonia and extract with four lots of ether, distil off the ether and dry the residue at 120°.

The product is colourless, odourless, free from iodine, and gives almost theoretical Gordin's titration values. It may be either weighed or estimated by titration.

For less accurate estimations the preliminary defaecation may be omitted, as no serious emulsion trouble arises when the solution of the precipitate brought down by Wagner's reagent is shaken up with ether.

The following Table shows the results obtained:—

c.c. of urine taken	Mgm. quinine added	Mgms. of quinine found by weight	Mgms. of quinine estimated by Gordin's titration
100	...	152	151·2
100	...	228	225·8
100	...	175	174·3
100	150	151	150·2
1000	100	102	98·8
100	100	100	98·7
100	100	101·6	99·6
1000	100	101	99·6
1000	100	101	98·8
1000	100	99·4	99·2
100	100	101	100·1
100 (water)	100	100	98·0
1200	100	102·2	97·3
100	100	101·6	96·8

The last two results quoted were obtained without preliminary defaecation of the urine.

NEPHELOMETRIC ESTIMATION OF QUININE

By the following procedure estimations of fractions of a milligram from 1/100th of a milligram upwards can be made with an error of less than 5 per cent.

It has been found invaluable in dealing with such small amounts of quinine as are contained in 5 c.c. of human blood when other methods would almost invariably be useless. It has also by reason of its rapidity proved very useful for approximate estimation of quinine in urine. Its success is due (1) to the great delicacy given to the Tanret turbidity test (*a*) by using purified ether (*b*) by dissolving the quinine in saturated solution of ammonium sulphate; (2) to the observation by one of us that comparisons of the opalescence of two suspensions can be made with greatly increased accuracy when the tubes are illuminated by a band of light admitted through a narrow adjustable slit. The alkaloid, extracted by a suitable process from the blood or urine so that it is free from substances which give Tanret turbidity, is introduced in ethereal solution into one of a special series of colourless, thin-walled glass test-tubes of about 13 mm. diameter. These tubes are carefully selected so that all are equal in diameter both internally and externally so far as the lowest 2 centimetres of the cylindrical portions are concerned. The external gauging is done with a smoothly bored cork, the internal with a piece of glass tubing on the roughened lowest half centimetre of which a piece of thick black rubber tubing has been firmly cemented by rubber solution. The ether is carefully evaporated off in a water bath, and the residual quinine is dissolved in 10 c.c. of saturated solution of ammonium sulphate. As this will dissolve only about 1·3 mgm. of quinine, the volume of urine or blood used must be chosen so that not more than this amount (for safety's sake, say, 0·8 mgm.) is obtained. Using 5 to 10 c.c. of human blood there is, in our experience, no danger of getting as much as this. Transfer 5 c.c. of the solution to another of the gauged test-tubes. Into five other test-tubes of the series introduce 5 c.c. of various standard solutions of quinine in saturated solution of ammonium sulphate of concentrations increasing from 1 mgm. in 500 c.c. to 1 in 100 c.c. To each of the six test-tubes 0·05 c.c. of Tanret's solution is then

added and thoroughly mixed at once. After a quarter to half an hour the tube is 'matched' with the standards in the special nephelometer, whereby an approximate idea of the concentration of the quinine is acquired. If this is less than 1 mgm. in 500 c.c. the turbidity will be too slight for any but very rough estimation: if more than 1 mgm. in 100 c.c., dilute the remaining solution of quinine with twice its volume of saturated ammonium sulphate solution, take 5 c.c. of the diluted solution and match with a series of fresh standards. The concentrations at which we find our judgments of turbidity most accurate, using 13 mm. test-tubes, lie between 1 mgm. in 200 and 1 in 300 c.c. The final matchings are made with a series of standard quinine solutions whose strengths decrease by tens from 1 in 200 to 1 in 300 c.c.

The nephelometer may be constructed in various simple ways; the one we have made consists of an oblong wooden box 24 centimetres long and 12 centimetres square in cross section, open at one of the narrow ends, and having the lower 10 to 12 centimetres of the other replaced by a well-made ebony parallel ruler, the lower bar of which is screwed firmly to the box. The upper bar being free, the width of the slit can be readily varied. By means of cardboard strips, attached to the box outside, light is blocked off from all but the central part of the slit. Outside the ruler and the cardboard screens is a piece of opal glass. The source of light is a partly-screened incandescent lamp. The roof of the box is perforated with an oblong aperture, of which the long side nearest the light is in line with the inner surface of the parallel ruler. The test-tubes are introduced two at a time through the roof aperture, and rest near together on plasticine (in the open ends of two partial rings of rubber tubing cemented to the floor of the box), so that their sides lie in *close contact with the ruler*. The upper ends of the tubes are held in position by sliding up against them a two-notched piece of thick felt. Observations should be made in a dark room or cupboard, and the observer should be sufficiently remote from the tubes to get full binocular vision.

We are not in a position to compare the results obtained by this apparatus with those obtainable by a plunger colorimeter of the Duboscq type. We doubt, however, whether any such instrument could serve our purpose. We have observed that the opalescences

of two solutions of known quinine concentration plus Tanret's reagent do not become identical when the stronger one is subsequently equalised with the weaker one (by dilution with $(NH_4)_2 SO_4$ plus Tanret's solution), a fact which indicates that the size of the turbidity particles, and therefore their quantitative effect in producing opalescence, depends largely on the concentration of the quinine solution when the reagent is added. If this is the case, their relative concentrations would not be accurately judged if assumed to be inversely proportional to the heights of the columns which show equal opalescence. To us it seems that the 'unknown' solution can only be matched accurately with a similar solution of identical initial concentration chosen empirically.

NEPHELOMETRIC ESTIMATIONS OF PURE QUININE

The alkaloid was given dissolved in a saturated watery solution of ammonium sulphate, 5 c.c. of the solution was taken for each estimation.

Number of c.c. containing 1 mgm. of quinine	Found	Quinine given, mgms. in 5 c.c.	Found	Percentage error
250	260	0·02000	0·01923	− 3·9
225	230	0·02222	0·02174	− 2·2
215	220	0·02326	0·02273	− 2·3
225	230	0·02222	0·02174	− 2·2
260	265	0·01923	0·01887	− 1·7
250	250	0·02000	0·02020	+ 1·0
200	192	0·02500	0·02538	+ 4·2

The observers did not know the correct results beforehand. Those shown above were obtained in a preliminary trial with test-tubes gauged only externally. We have no doubt they can be improved on, and also that with suitable flat-bottomed tubes 2·5 c.c. of solution would suffice, in which case it would be possible to estimate 1/200th mgm. of quinine with the same degree of accuracy.

NEPHELOMETRIC ESTIMATION OF QUININE IN BLOOD

A known volume of blood (5 to 10 c.c.) is removed from the patient by a syringe and ejected at once into a clean flask containing 2·5 to 5 grams of solid ammonium sulphate and 10 c.c. of a saturated solution of this salt containing 0·6 per cent. H_2SO_4. Boil for two minutes, shaking all the time, allow to settle, pour off the liquid and filter while hot through a small Gooch crucible under suction. Catch the filtrate in a test-tube and transfer it to a 50 c.c. stoppered cylinder. Add 10 c.c. more acidulated ammonium sulphate solution to the original flask, boil and filter as before. Wash the residue on the crucible twice with the hot filtrate before adding it to the original filtrate. Repeat the boilings with three further lots of $(NH_4)_2SO_4$ solution as before, on each occasion washing the residue on the crucible two or three times. Cool the filtrate, alkalise with ammonia and extract with four 7 c.c. lots of purified ether, removing each lot as it separates (it is absolutely colourless) and transferring it to one of the gauged test-tubes. Evaporate off the ether in a hot-water bath, taking care to avoid loss by spurting—the tube now contains the whole of the quinine of the original blood. Control experiments with normal blood have shown that substances other than quinine which give a turbidity with Tanret's reagent are completely absent. Numerous other procedures were tried, but none fulfilled our requirements.

Since the appearance of Teichman's paper we have repeated on *normal* blood his procedure for extracting quinine. Diluted blood was made alkaline with sodium hydrate and then shaken up with ether. The ether separated slowly; it was yellow with extracted pigment, and the residue left on its evaporation gave when dissolved in 1 c.c. of 0·04 per cent. hydrochloric acid a marked turbidity with Tanret's reagent. This cleared up on addition of two drops of strong hydrochloric acid—presumably it is in order to eliminate error from this impurity that Teichmann adds strong acid, although by so doing so he greatly diminishes the delicacy of the test (*vide supra*).

Known amounts of anhydrous quinine alkaloid dissolved in dilute hydrochloric acid were mixed with defibrinated sheep's blood and given to the observer. 5 c.c. of the mixture was used for each estimation.

Concentration given	Concentration found	Quinine given, Mgms. in 5 c.c.	Found	Error
1 in 166,666	1 in 160,000	0·03030	0·03125	+ 3·1
1 in 250,000	1 in 260,000	0·02000	0·01923	− 3·8
1 in 250,000	1 in 255,000	0·02000	0·01961	− 2·0
1 in 286,000	1 in 290,000	0·01786	0·01724	− 3·5

We may mention that we have found as much as 0·040 mgms. of quinine in 5 c.c. of the blood of a non-malarial patient taking 5,950 mgms. of alkaloid (= 90 grains) per diem. During the period when the blood steadily maintained the above concentration of 8 mgms. of quinine per litre, some of the urine secreted had as much as 3,000 mgms. per litre!

Such clinical estimations of quinine in blood as we have made up to the present suggest that the concentrations attained in chronic malarial subjects are always much lower than in healthy men taking the same dose of the drug in the same way. The question arises whether these lower concentrations are due to 'habituation' to quinine (cf. Teichmann), or are from the very beginning characteristic of men who, if infected with malaria, will tend to relapse after relapse, because, owing to some individual idiosyncrasy, a certain minimal concentration of quinine necessary in the blood for complete eradication of the parasite will never be attained. It is a question the solution of which might well afford important indications for prophylaxis and treatment. Now that an adequate method of estimation is available, we hope that other workers who, unlike ourselves, may have access to fresh cases of malaria not previously 'habituated' to quinine will contribute to its solution by estimating the blood-quinine in as many of them as possible.

NEPHELOMETRIC ESTIMATION OF QUININE IN URINE

To every 100 c.c. of urine add 5 c.c. of 25 per cent. solution of neutral acetate of lead containing also $2\frac{1}{2}$ per cent. of acetic acid. Mix and then add 5 c.c. of saturated watery solution of ammonium sulphate. Filter until a clear filtrate is obtained. Test it for

absence of lead by receiving a drop on filter paper moistened with a sulphide solution. If lead is found, the defaecation process must be omitted. Reckon 100 c.c. of filtrate as representing 92·3 c.c. of the original urine.

Test the filtrate with Tanret's reagent, and if it gives any turbidity dilute it with known volumes of water until it just ceases to do so.

Take 10 c.c. of this 'defaecated' (and, if necessary, diluted) urine in a small stoppered cylinder and dissolve in it 5 grams of ammonium sulphate. Extract with three successive 7 c.c. lots of purified ether, and so get rid of any 'oily matter.' Alkalise it with strong ammonia solution and extract the quinine with three successive lots of ether, transferring each lot as removed to one of the gauged test-tubes. Then proceed as described for nephelometric estimation.

NEPHELOMETRIC ESTIMATIONS OF URINE

Quinine given, mgms. in 10 c.c.	Found	Percentage error
0·1000	0·0994	− 0·5
0·0588	0·0552	− 6·1
0·0833	0·0832	− 0·2
0·1205	0·1232	+ 2·2
0·0400	0·0401	+ 0·2

APPENDIX

Christensen's Reagent for Herapathite (far better than Jörgensen's or Autenrieth's)

Iodine, 1 gram.
50 per cent. hydriodic acid, 1 gram.
Strong H_2SO_4, 0·8 gram.
70 per cent. alcohol, 50 grams.

Tanret's Reagent

Dissolve 1·35 grams of mercuric perchloride in 75 c.c. of water, and 5 grams of potassium iodide in 20 c.c. of water in a 100 c.c. measuring flask. Pour the mercuric solution into the iodide solution under agitation and fill up with water to the mark.

Giemsa and Schaumann's Reagent

= Tanret's reagent plus 1 per cent. glacial acetic acid.

Wagner's Reagent

Iodine, 1 gram.
Potassium iodide, 1·5 gram.
Water up to 100 c.c.

Purification of Ether

One litre of methylated ether is shaken up thoroughly for five minutes with four successive 100 c.c. lots of saturated aqueous solution of sodium bisulphite in a separating funnel, allowing half an hour for each separation before running out the watery solution. Wash with 50 c.c. of half-saturated NaCl solution. The ether is then shaken with 50 c.c. of water plus a little phenol-phthalein and enough sodium hydrate solution to make it alkaline. The separated ether is further purified by distillation. It must give no reaction for aldehydes or ketones, otherwise Tanret turbidity with the residue of an ether extract will be inconclusive, and any quinine obtained will be impure.

As a supremely delicate test for ketones the mercuric cyanide reagent of Scott-Wilson can be strongly recommended. As a test for aldehyde, though a much less sensitive one, the well-known Schiff's sulphurous acid fuchsin solution may be used.

The ultimate criterion should be the Scott-Wilson reagent—the reagent must be added in excess.

Scott-Wilson Reagent

Mercuric cyanide	0·5 gram.
Sodium hydrate	9·0 gram.
Water	60·0 gram.
Silver nitrate solution (0·7268 per cent.)...	20·0 gram.

The soda and mercuric cyanide are dissolved in the water and then the silver solution is run in while constantly stirring.

SUMMARY

1. Procedures are described which greatly increase the delicacy of the Thalleioquin and the Herapath tests for quinine.

2. The turbidity given by Tanret's reagent can be made an exceedingly delicate 'negative test' for quinine, and, with adequate precautions for excluding other substances, into a positive test capable of detecting 1/5000th mgm.

3. A defaecation process is described for urine which removes no quinine but abolishes ether emulsion troubles, and usually also removes all substances other than quinine which give Tanret turbidity.

4. Commercial ether cannot safely be used for extraction of quinine, whether for qualitative or quantitative purposes, without special purification. An adequate and simple method for such purification is given.

5. The accuracy of the Gordin volumetric method for the estimation of quinine is supported.

6. Giemsa and Schaumann's method for estimating quinine in urine has certain defects. It is shown how these may be diminished.

7. A new method of extracting quinine from urine is described—time, labour and material are saved, and the quinine obtained is so pure that it can be estimated by titration.

8. By the use of an adjustable slit to secure variable and equal illuminations greatly increased accuracy is attainable in nephelometry.

9. A new method for nephelometric estimation of quinine in blood is described, delicate enough for use with a few c.c. of human blood. The same method serves also for rapid estimations of quinine in urine.

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Vol. XI. No. 1. June, 1917

Observations on Malaria in the Gold Coast Colony, West Africa. By J. W. S. MACFIE, D.Sc., M.B., and A. INGRAM, M.D. Two plates.

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The Occurrence of *Ankylostoma ceylanicum* in West African Dogs. By WARRINGTON YORKE, M.D., and B. BLACKLOCK, M.D., D.P.H.

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The Relapsing Fever Spirochaetes. By J. W. S. MACFIE, D.Sc., M.B., and WARRINGTON YORKE, M.D.

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Studies in the Treatment of Malaria: I.—Intravenous Injections of Tartar Emetic. By LT.-COL. J. W. W. STEPHENS, M.D., D.P.H., R.A.M.C., WARRINGTON YORKE, M.D., B. BLACKLOCK, M.D., D.P.H., J. W. S. MACFIE, D.Sc., M.B., and C. FORSTER COOPER.

Studies in the Treatment of Malaria: II.—Intramuscular Injections of Quinine Bihydrochloride in Simple Tertian Malaria. By LT.-COL. J. W. W. STEPHENS, M.D., D.P.H., R.A.M.C., WARRINGTON YORKE, M.D., B. BLACKLOCK, M.D., D.P.H., J. W. S. MACFIE, D.Sc., M.B., and C. FORSTER COOPER.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY

Vol. XI. No. 2. August, 1917

Observations on the Periodicity of *Microfilaria nocturna*. By WARRINGTON YORKE, M.D., and B. BLACKLOCK, M.D., D.P.H. One plate.

Studies in the Treatment of Malaria: III.—Intravenous Injections of Quinine Bihydrochloride. By LT.-COL. J. W. W. STEPHENS, M.D., D.P.H., R.A.M.C., WARRINGTON YORKE, M.D., B. BLACKLOCK, M.D , D.P.H., J. W. S. MACFIE, D.Sc., M.B., and C. FORSTER COOPER.

Studies in the Treatment of Malaria: IV.—Intramuscular Injections of Amylopsin and Trypsin in Simple Tertian Malaria. By LT.-COL. J. W. W. STEPHENS, M.D., D.P.H., R.A.M.C., WARRINGTON YORKE, M.D., B. BLACKLOCK, M.D., D.P.H., J. W. S. MACFIE, D.Sc., M.B., and C. FORSTER COOPER.

Studies in the Treatment of Malaria: V.—Intramuscular Injections of Quinine Alkaloid in Simple Tertian Malaria. LT.-COL. J. W. W. STEPHENS, M.D., D.P.H., R.A.M.C., WARRINGTON YORKE, M.D., B. BLACKLOCK, M.D., D.P.H., J. W. S. MACFIE, D.Sc., M.B., C. FORSTER COOPER, and H. F. CARTER, F.E.S.

Further Records of the Occurrence of Intestinal Protozoa in Non-Dysenteric Cases. By A. MALINS SMITH, M.A., and J. R. MATTHEWS, M.A.

The Value of Concentrating the Cysts af Protozoal Parasites in Examining the Stools of Dysenteric Patients for Pathogenic Entamoebae. By HENRY F. CARTER, F.E.S., and J. R. MATTHEWS, M.A.

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ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY

Vol. XI. No. 3. January, 1918

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Studies in the Treatment of Malaria: VI.—Oral Administration of Quinine for two consecutive days only in Simple Tertian Malaria. LT.-COL. J. W. W. STEPHENS, M.D., D.P.H., R.A.M.C., WARRINGTON YORKE, M.D., B. BLACKLOCK, M.D., D.P.H., J. W. S. MACFIE, D.Sc., M.B., C. FORSTER COOPER, and H. F. CARTER, F.E.S.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY

Vol. XI. No. 4. May, 1918

Studies in the Treatment of Malaria: VII.—Oral Administration of Quinine Sulphate daily over prolonged periods, in Simple Tertian Malaria. LT.-COL. J. W. W. STEPHENS, M.D., D.P.H., R.A.M.C., WARRINGTON YORKE, M.D., B. BLACKLOCK, M.D., D.P.H., J. W. S. MACFIE, D.Sc., M.B., C. FORSTER COOPER, and H. F. CARTER, F.E.S.

Studies in the Treatment of Malaria: VIII.—Oral Administration of Quinine Sulphate for two consecutive days weekly over prolonged periods, in Simple Tertian Malaria. LT.-COL. J. W. W. STEPHENS, M.D., D.P.H., R.A.M.C., WARRINGTON YORKE, M.D., B. BLACKLOCK, M.D., D.P.H., J. W. S. MACFIE, D.Sc., M.B., C. FORSTER COOPER, and H. F. CARTER, F.E.S.

Studies in the Treatment of Malaria: IX.—A Comparison of the Results of Interrupted and Continuous Quinine Administration. LT.-COL. J. W. W. STEPHENS, M.D., D.P.H., R.A.M.C., WARRINGTON YORKE, M.D., B. BLACKLOCK, M.D., D.P.H., J. W. S. MACFIE, D.Sc., M.B., C. FORSTER COOPER, and H. F. CARTER, F.E.S.

A Comparative Study of the Habits of *Glossina brevipalpis*, Newst., *G. fusca*, West., and *G. pallidipes*, Aust., in the Belgian Congo. Dr. SCHWETZ. One map and one chart.

***Strongylidae* in Horses: *I.—Cylicostomum longibursatum* sp. n.** WARRINGTON YORKE, M.D., and J. W. S. MACFIE, D.Sc., M.B.

***Strongylidae* in Horses: *II.—Cylicostomum minutum* sp. n.** WARRINGTON YORKE, M.D., and J. W. S. MACFIE, D.Sc., M.B.

***Strongylidae* in Horses: *III.—Cylicostomum nassatum*, Looss. var. *parvum*.** WARRINGTON YORKE, M.D., and J. W. S. MACFIE, D.Sc., M.B.

Studies in the Treatment of Malaria: X.—Oral Administration of Quinine Sulphate Grains 120 on two consecutive days only, in Simple Tertian Malaria. LT.-COL. J. W. W. STEPHENS, M.D., D.P.H., R.A.M.C., WARRINGTON YORKE, M.D., B. BLACKLOCK, M.D., D.P.H., J. W. S. MACFIE, D.Sc., M.B., C. FORSTER COOPER, and H. F. CARTER, F.E.S.

Studies in the Treatment of Malaria: XI.—Oral Administration of Quinine Sulphate Grains 90 on two consecutive days weekly over a period of three weeks, in Simple Tertian Malaria. LT.-COL. J. W. W. STEPHENS, M.D., D.P.H., R.A.M.C., WARRINGTON YORKE, M.D., B. BLACKLOCK, M.D., D.P.H., J. W. S. MACFIE, D.Sc., M.B., C. FORSTER COOPER, and H. F. CARTER, F.E.S.

Studies in the Treatment of Malaria: XII.—At what time after cessation of Quinine Treatment do Relapses occur in Simple Tertian Malaria? LT.-COL. J. W. W. STEPHENS, M.D., D.P.H., R.A.M.C., WARRINGTON YORKE, M.D., B. BLACKLOCK, M.D., D.P.H., J. W. S. MACFIE, D.Sc., M.B., C. FORSTER COOPER, and H. F. CARTER, F.E.S.

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